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Anatomical development in soybean hypocotyls sensitive to temperature-dependent growth anomaly

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Anatomical development in soybean hypocotyls sensitive to temperature-dependent growth anomaly

by

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INTRODUCTION

The inhibition of hypocotyl elongation accompanied by increased radial expansion in certain cultivars of soybean [Glycine max (L.) Merr.) at 25 C was first reported by Grabe and Metzer (1969). Their research was initiated in response to reports by Iowa soybean producers of emergence problems encountered when these "short hypocotyl" cultivars were planted in the field.

Since that time several other researchers have studied the physiological, agronomic, and genetic aspects of this problem (Samimy, 1970; Knittle, 1977; Keys, 1979; Seyedin, 1981; Fehr, 1973). These studies were conducted to elucidate the controlling mechanisms for the response, including the involvement of ethylene and auxin, and the effects of physical resistance on hypocotyl elongation in cultivars possessing the 25 C anomalous growth pattern.

The present study was initiated to characterize the anatomical features of the soybean hypocotyl which may be affected in this process. These features include the cell number and cell size of the cortex and pith regions, and size of the vascular cylinder. Of particular interest was the contribution of these portions of the hypocotyl anatomy to the increase in radial expansion, and the timing of changes in elongation/expansion in response to temperature. To aid

in describing the changes from normal growth occurring in short hypocotyl types, these cultivars are compared with "long hypocotyl" cultivars that respond linearly to increasing temperature.

LITERATURE REVIEW

Several factors affect the expression of emergence potential of crop seed. These include environmental factors such as soil moisture, aeration, compaction, temperature, pathogens, and light. Bowen and Hummel (1980) listed the two most important environmental factors as temperature and impedance. Plant factors such as species, cultivar, seed quality, seed size, plant growth regulator effects, and interactions between the various factors are also important considerations. The purpose of this literature review is to survey the known effects of environmental and plant factors influencing elongation of seedling emergence organs and subsequent stand establishment, the relationship between field emergence and laboratory evaluations of seedling vigor, and literature specific to the temperature-induced inhibition of hypocotyl elongation in certain soybean cultivars at 25 C.

Environmental Effects on Seedling Emergence

Temperature

Temperature generally has its effect on seedling emergence by influencing the rate of elongation of the plant organ responsible for pushing the future above-ground parts of the plant through the soil surface. Temperature also influences germination which occurs prior to growth of the

emerging organ. Cardwell (1984, p. 68) lists optimum emergence temperatures for 17 crop species. Equations developed by Hatfield and Egli (1974) to predict time to 50% emergence indicate that the optimum time to plant soybeans is when the soil temperature is between 25 and 35 C, if other factors are not limiting. They also reported that the rate of hypocotyl elongation was increased and time to 50% emergence was shortened by increasing temperature from 10 to 30 C. Razera (1982) reported a range for optimum emergence of soybean between 28 and 32 C. Soybean emergence from a soil-sand-peat mixture was increased at three successively higher temperatures: 16, 24, and 32 C (Stucky, 1976). Hopper et al. (1979) reported that time to 50% emergence decreased from 18.8 to 4.0 days as temperature was increased by 5 C increments between 10 and 30 C and did not decrease between 30 and 35 C. Simulated conditions of later planting dates from 16 April to 15 June, and the expected warmer soil temperatures, increased speed of emergence from the soil-sand-peat mixture. Cultivar differences in emergence rate were not detected at temperatures below 20 C. Goyal et al. (1980a, 1980b) reported that soybean seedling emergence force, measured with a force transducer, increased from 2.92 to 3.75 N and that the critical time (time after planting until maximum force is achieved) decreased when temperature was increased from 15 to 32 C.

Imbibitional chilling injury reduces germination and seedling growth by damaging the embryonic axis. Ashworth and Obendorf (1980) observed stelar lesions in the hypocotyls of soybean seedlings which had undergone imbibitional chilling injury. These lesions were prevalent when soybeans initially at 6% moisture were imbibed at 5 C. However, preconditioning seed to 17% moisture before rapid imbibition seemed to protect embryos from such injury as there was less shock to membranes.

Williams (1963a) reported the optimum temperature for maximum emergence force of crimson clover (Trifolium incarnatum L.) to be 20 C, and that of alfalfa (Medicago sativa L.) to be 30 C. Sprague (1943) reported that in several grass and forage legume species seedling establishment was generally best at temperatures between 55 and 70 F (13 and 21 C) and was generally poor above 85 F (29 C). A similar optimum temperature range to that reported for soybean was reported for cotton (Gossypium hirsutum L.) by Krzyzanowski (1980). An increase in the rate of elongation of cotton hypocotyls and radicles with increase in temperature from 15.6 to 32.2 C was reported by Wanjura and Buxton (1972a). Arndt (1945) reported that the temperature optimum for cotton hypocotyl elongation shifted from about 33 C at planting to about 36 C four to five days after planting, illustrating the adaptation of seedlings to the changing

microenvironment as it approached the soil surface. The temperature optimum for primary root growth shifted downward from 33 to 27 C over the same time period.

Pollock (1962) reported that "physiological dwarfing" in peach [Prunus persica (L.) Batsch cv. Elberta] seedlings occurs at 25 C but not at 19 or 22 C or in the presence of an alternating 19-25 C temperature regime. Seedlings appear to be sensitive to this temperature response until about nine days after planting. The severity of symptoms but not the time period involved was lessened by an afterripening treatment at 5 C prior to planting. Pollock discounted the idea that the primary influence was the removal of a growth inhibitor by the afterrippening treatment. Instead, he suggested that the dwarfing condition was due to a temperature-sensitive conversion of meristem cells to the dwarfed condition which was perpetuated in the seedling through division of affected cells and their daughter cells, and was more severe as a result of longer exposure to 25 C. This condition is probably due to a different physiological mechanism than the inhibition of hypocotyl elongation in soybeans, although it does occur at the same temperature.

Models to predict emergence of wheat (Triticum aestivum L.) and corn (Zea mays L.) as a function of temperature have been developed. Lindstrom et al. (1976) reported a decrease in emergence rate as temperature was reduced from 25 to 5 C

in a model including temperature, soil moisture potential, and planting depth. Regardless of soil water potential or depth of planting, time to 50% emergence was reduced from six weeks at 5 C to two weeks at 12.2 C, and to within one week at 19.4 and 26.7 C (De Jong and Best, 1979). Blacklow (1972) reported that rate of mesocotyl elongation in corn was greatest at 30 C and ceased below 9 or above 40 C. Blacklow (1973) later developed a simulation model for corn that indicated that corn seedlings responded to the prevailing temperature regime with no adaptation to preceding conditions and that the response to changing temperature occurred within minutes. However, the model was not useful under stressfully high or low temperatures or if other soil factors were limiting.

Moisture

Another environmental factor which influences seedling emergence and interacts with temperature is soil moisture. Adequate but not excessive moisture is required for germination and emergence of seed. The effects of temperature reported by Hatfield and Egli (1974) and Stucky (1976) for soybeans hold if moisture is not a limiting factor. In cotton, increased soil moisture tension (3.0 to 0.3 bars [0.3 to 0.03 MPa]) consistently resulted in decreased hypocotyl elongation and increased radicle

elongation (Wanjura and Buxton, 1972a). Cotton hypocotyl elongation was completely inhibited at 8 to 10 bars (0.8 to 1 MPa) moisture tension. Water uptake rate increased as temperature increased and soil moisture tension decreased (Wanjura and Buxton, 1972b). In sugar beet (Beta vulgaris L.), final germination percent was not changed. Germination rate; however, decreased under simulated moisture stress between 0 and -4 bars (0 and -0.4 MPa) (Akeson et al., 1980). Germination rate and final percent germination decreased at moisture stresses between -4 and -8 bars (-0.4 and -0.8 MPa). Germination of sugar beets, especially low germinating seed lots, was improved under these moisture stress conditions by removal of the pericarp or treatment with 1.0 N HCl. Soil moisture stress due to high salinity was reported to reduce germination percentage and rate in sugar beet and onion (Allium cepa L.). The toxic effects of the salt may; however, have accounted for some of the injury (Ayers, 1952).

In Lindstrom et al.'s (1976) wheat emergence model the minimum moisture potential necessary for emergence to proceed was increased with increasing temperature, and the practical lower limit for field emergence of wheat was between -6 and -7 bars (-0.6 and -0.7 MPa). De Jong and Best (1979) reported that the minimum temperature for emergence was reduced from 1.3 to 0.2 C as soil water potential decreased from $-^{1}/_{3}$ to -10 bars (-0.03 to -1 MPa). Wheat germination

was not affected by moisture stress up to 12 atm (1.2 MPa) but was significantly reduced between 15 and 18 atm (1.5 and 1.8 MPa) (Ashraf and Abu-Shakra, 1978).

Physical impedance

An emerging seedling must exert an upward force greater than the opposing force of soil resistance in order to emerge. Resistance to seedling penetration by the soil may be increased if the soil is heavily compacted or if the soil surface is crusted (Arndt, 1965a). Soil physical properties which contribute resistance to seedling emergence include density, soil cohesion, and friction between soil structural units (Arndt, 1965b). Emergence force has been estimated in the field by varying planting depth (Stucky, 1976; Lindstrom et al., 1976), artificial compaction with some heavy object (Knittle et al., 1979), or by creating a crusted surface using simulated rainfall (Arndt, 1965b). Emergence force of seedlings has been measured in laboratory situations by placing weights on top of seedlings (Williams, 1963a; Knittle and Burris, 1979b; Howle and Caviness, 1988), then calculating the force exerted by the seedling to move a given weight, or by direct measurement of the force exerted in soil or some other medium by means of a force transducer (Taylor and Ten Broeck, 1988; Gerard, 1980; Johnson and Henry, 1967; Goyal et al., 1980a, 1980b). Taylor and Ten Broeck (1988)

reported individual seedling forces ranging from 26 mN for table beets (Beta vulgaris L.) to 3400 mN for snap beans (Phaseolus vulgaris L.). Goyal et al. (1980a) reported a maximum value for soybean seedling emergence force of 3.88 N. The maximum emergence force in cotton measured by Gerard (1980) was 600 g (6.0 N) at 27 C.

Knittle and Burris (1979a) described the effects of downward force on soybean hypocotyls. The downward force was supplied by weights placed on top of the growing seedlings enclosed in a piston apparatus. An increase in downward force resulted in a decrease in hypocotyl length and an increase in hypocotyl swelling index (mg fresh weight/cm length). These responses appeared to be independent of temperature and were functions of cultivar and seed size within cultivar. Temperature affected hypocotyl elongation rate as previously discussed. Victor and Vanderhoef (1975) reported radial enlargement of excised sections of elongating hypocotyls subjected to physical impedance in the presence of exogenous cytokinin and auxin on the order of nine times that of unimpeded controls.

In response to physical impedance, thickening of the hypocotyl should increase the strength of the hypocotyl, and increase its ability to break through the soil barrier (Knittle and Burris, 1979a; Gerard, 1980); however, hypocotyl swelling in response to physical resistance may not be via

the same mechanism as that in the 25 C hypocotyl growth anomaly (Knittle and Burris, 1979a). Then tendency of a column to buckle under pressure increases as the length of the column increases if there is no proportional increase in diameter. The lack of a large cross-sectional area in the cotton hypocotyl, combined with increasing length, was reported to reduce the effective axial force of the hypocotyl and, therefore its ability to overcome soil impedance, especially in a soil of high impedance (Arndt, 1965a).

Pathogens

A major effort of many plant breeding programs is disease resistance against seed- and soil-borne organisms which interfere with normal seedling emergence and stand establishment. New cultivars of crop plants are often developed, and older cultivars improved through backcrossing with resistant genotypes, to improve resistance to a particular pathogen or races of a pathogen. Increased Phytophthora root rot resistance was the primary improvement of the 'Amsoy 71' (Probst et al., 1972), 'Beeson 80' (Wilcox et al., 1980), 'Corsoy 79' (Release notice obtained from Dr. G. E. Pepper, Department of Agronomy, Univ. of Illinois at Urbana-Champaign), and 'Oakland' (Bahrenfus and Fehr, 1980) cultivars used in the present study compared to the cultivars they were intended to replace. Phytophthora spp., Pythium

spp., Rhizoctonia solani, and Sclerotinium, among others, infect seedlings and cause stand reductions. R. solani was reported to delay emergence of soybeans by inhibiting hypocotyl elongation when seed coats, the source of inoculum, remained attached to the seedling (Grau and Martinson, 1979). Greatest reductions in hypocotyl elongation of soybeans infected with R. solani occurred at 25 C. No reductions were reported at 30 C. In addition to inhibited elongation, hypocotyls were swollen and had brown discolorations (Grau, 1971).

Prediction of Field Emergence by Laboratory Tests

The problem of using laboratory evaluations of seed quality in predicting field emergence has been approached in several ways. Visual ratings such as wrinkled seed coats, shriveled cotyledons, green cotyledons, and overall appearance were used to select lines for advancement in breeding for field emergence (Green et al., 1965) and were about as effective for that purpose as laboratory germination tests. An average rating of these visually determined factors was generally better than any single visual rating in predicting field emergence (Green and Pinnell, 1968b).

Tekrony and Egli (1977) reported that the standard germination test, an accelerated-aging test, a four-day germination test, and vigor indices derived from the results

of these tests tended to overestimate field emergence especially under adverse field conditions. Johnson and Wax (1978) reported that the cold test was better correlated with field emergence and, therefore more reliable in identifying seed lots which would have emergence problems under adverse field conditions than standard germination or accelerated aging tests. Accelerated aging was reported to reduce vigor in high quality soybean seed lots resulting in decreased field emergence, but the effects of accelerated aging were not found to influence growth or yield parameters of established stands (Edje and Burris, 1971). Various indices of seedling vigor were compared by Burris et al. (1969). Seven-day germination tended to overestimate seedling vigor. Cold test, 4-day germination, and growth rate (mm/day) were reported to be better estimates of vigor. Glucose levels in dry seed and respiration rate of imbibed seed correlated with vigor tests more closely than soluble amino acid content, glutamic acid decarboxylase activity, or reducing sugar level. The best estimate of seedling vigor in this study was given by the ratio of 4-day to 7-day germination because it reduced the effect of cultivar variation. A packed sand test was reported to be better correlated than standard germination in predicting field emergence of sugar beet (Akeson and Widner, 1980). In cotton, time to 50% emergence plus total emergence was reported to be a better predictor of

predictor of seedling emergence than field emergence plus percent transfer (ratio of plant axis weight to total seedling weight) (Buxton et al., 1977).

Various methods of laboratory evaluation of seedling vigor were compared by Burris and Fehr (1971). It was their view that planting in sand resulted in greater differences between long and short hypocotyls because of the increased physical resistance; however, rolled towels provided a more efficient method where large numbers of samples were involved or where measurement of individual hypocotyls was desired. Hypocotyl breakage was shown to be detectable in Amsoy 71 at about day five at 25 C when grown in rolled towels but was not apparent when grown on Kimpak (Seyedin, 1981).

Knittle et al. (1979) developed regression equations for hypocotyl elongation using field data. Two equations were developed to predict the hypocotyl elongation rate for the two phases of seedling development: growth phase and elongation phase, the division between the two phases being 200 hr after planting. The regression equation for the growth phase, which would include imbibition and radicle protrusion, predicts that hypocotyl elongation rate would increase with increasing temperature and soil moisture. In the elongation phase, the regression equation predicts that as soil resistance increases, hypocotyl elongation decreases with a significant temperature X resistance interaction also

causing a decrease in elongation.

Plant Factors Influencing Emergence

Seed size

Species of epigeal emergence account for approximately 90% of the dicotyledonous species grown as crops (Nelson and Larson, 1984). Epigeal emergers must manage to get the two cotyledons above the soil surface before the stored reserves are depleted. The cotyledons themselves may contribute to the problem because of their size. In large seeded dicots such as soybean the cotyledons comprise most of the bulk of the seed and largely determine seed size (Black, 1956). Seed size may then be an important factor in seedling emergence and in hypocotyl elongation in two ways: by supplying assimilates to the growing region of the hypocotyl (Wahab and Burris, 1975a,b) and by being the organs which meet the resistance of the soil directly.

Seed size effects on hypocotyl elongation and seedling emergence have been studied in several ways with some conflicting reports. Burris et al. (1971) reported no significant cultivar differences between 'Amsoy', 'Wayne', 'Hawkeye', and 'Corsoy' grown at 25 C in rolled towels, but reported that as seed size decreased there was an increase in shoot growth and a decrease in radicle growth. Johnson and Luedders (1974) reported no effects attributable to seed size

on emergence or yield in four cultivars, each divided into three size classes. Burris et al. (1973) reported that large seed of Amsoy emerged better from, and had longer hypocotyls in 3 cm of sand than small seed. The seed size effect was reversed in 10 cm of sand presumably because smaller cotyledons of small seed encountered less resistance from the growing medium. Emergence and hypocotyl length were reduced in long hypocotyl cultivars Wayne and Hawkeye only for the smallest seed size tested. Under simulated soil crust conditions, large seed of 'Mack' and 'Hill' soybean generally had a greater percent emergence and root and shoot fresh weight than small or unsized seed lots (Longer et al., 1986).

Hoy and Gamble (1985) studied the combined effects of seed size and seed density on soybean seedling vigor. Large, low-density seed performed poorly in germination tests and had high single-seed conductivity values indicating low vigor. Differences associated with seed size and density were slight or nonexistent in seed lots of extremely high or extremely low vigor. Size and density effects are noted most often in seed lots of marginal quality. The benefit of removing low-density seed is more beneficial in such seed lots than in extremely high vigor or seed lots of such low vigor that conditioning costs would be prohibitive (Hoy and Gamble, 1987). Small seed were reported to have a lower emergence potential and the large, dense seed were superior

to the unsized seed lot. Howle and Caviness (1988) reported no clear association between seed weight or seed width and the ability of seedlings to displace a 65 g load at 25 C. Seed produced on the upper third of the plant exhibited greater displacement and were wider and heavier than seed produced lower on the plant. Seed from two-seeded pods displaced the weight better than seed from one-seeded pods. These results are somewhat conflicting since seed from one seeded pods should be larger and heavier than seed from two-seeded pods on the basis of assimilate distribution. Gupta (1976), using isogenic lines of 'Lee' soybean differing in seed size and seed lots sized by sieving, reported that "physiologically large" seed were of better quality than small seed, but genetic seed size variants could not be separated adequately by conductivity and normal vigor tests.

In snap beans, Clark and Peck (1968) reported that rows planted with large seed generally outyielded rows planted with small seed when the same number of seed per row were planted. When equal weights of seed were planted per row small seed outyielded large seed. Seed lots containing small seed generally had more seed with intact cotyledons, i.e., less mechanical damage, than large-seeded lots.

Seed size was reported to have an effect on the maximum emergence force of several forage legume species by Williams (1963a). He reported that differences in seed size accounted

for 53% of the variation in emergence force among the species. Seed weight was reported to account for 99% of the variation in maximum emergence force, as analyzed by probit analysis, in crimson clover, subterranean clover (Trifolium subterraneum L.), rose clover (Trifolium hirtum All.), and alfalfa (Williams, 1963b). In subterranean clover, dry weight at any point in the early vegetative stage was linearly related to embryo weight, and differences in early growth may be due to distribution within seed populations (Black, 1957). Superior emergence potential in white lupine (Lupinus albus L.) may be attained by selecting for lines with a seed size of at least 200 mg/seed (large for white lupine) and a high hypocotyl elongation rate (Long, 1982). Seed from F_4 lines from a cross of Vicia sativa x V. angustifolia with seed weights intermediate between the two parents had more rapid emergence and better final stands than either parent (Allen and Donnelly, 1966). Rate of emergence was the most important factor in field emergence of these seed despite the fact that heavier seed appeared more vigorous in greenhouse tests. Taylor and Ten Broeck (1988) reported that small seed were more efficient in utilizing reserve materials than large seed in nine vegetable crop species of varying seed sizes.

Inheritance of emergence potential

Some attempts have been made to determine the heritability of seed quality factors related to emergence and stand establishment. Green et al. (1971) reported that heritability for field emergence was lower than that for visually rated seed quality factors such as cracked or wrinkled seed coats, shriveled or green cotyledons, and overall visual appearance. The heritability of field emergence was low due to environmental variations, such as weathering, harvest moisture, etc. (Green et al., 1966). Such results led to the recommendation that improvement of soybean field emergence would be more likely to occur if selection pressure was applied after the F_3 generation (Green and Pinnell, 1968a). Howle (1984) reported that genetic control of hypocotyl elongation was by genes with variable and small effects, but did not mention a major gene effect. Only the ability to overcome resistance was determined. Most parts of the study were conducted at room temperature and light effects on hypocotyl elongation were not taken into account.

Plant growth regulators and tissue development

Growth of elongating plant organs and cells can be divided into two basic processes: surface extension and cell partitioning (Green, 1976). Surface extension includes any process which contributes to an overall increase in volume of

the tissue or cell. Cell partitioning as defined by Green is the completion of the cross wall creating two cells from one and is equivalent to cell division. Plant hormones and other substances have been variously reported to affect these two basic processes. Interaction between various classes of growth inducing and inhibiting compounds is common as are indirect and secondary effects. In addition, hormones may occur in various forms depending on species, tissue, and stage of plant development such as the variations described for auxins by Bandurski and Schulze (1977). Auxins and ethylene are most often discussed in regard to elongation of seedling organs, especially in legumes; however, other hormones such as cytokinins and gibberellins are also important.

Auxins have generally been reported to stimulate growth in elongating tissues. The Avena coleoptile elongation test is a standard bioassay for auxin activity (Sirois, 1966). Auxin was reported to reverse the inhibition of hypocotyl lengthening induced by red light in white lupine (Acton and Murray, 1974). Gibberellin did not affect the red-light induced inhibition but did reverse inhibition mediated by far-red light. Thomas and Raper (1985) reported, for soybean, that when photoperiod was increased by incandescent lighting (low red:far red ratio), petiole elongation increased and internode elongation decreased when given alone

or following fluorescent (high red:far red ratio) irradiation. Light inhibited growth in light and dark grown hypocotyls of green cress (Lepidium sativum L.). Phytochrome involvement is probable, as indicated by the return to rapid growth when etiolated plants were returned to darkness. When green seedlings were placed in darkness following a light treatment growth was not as rapid as before the light treatment (Gordon et al., 1982), although no specific phytochrome forms were identified. Kristie and Jolliffe (1987) reported that inhibition of elongation in Sinapis alba hypocotyls by blue light was stronger than that caused by red light, but was also more quickly reversed. Dai and Galston (1981) reported that red light simultaneously inhibited arginine decarboxylase (ADC) activity in epicotyls and stimulated ADC activity in buds. Both effects were reversed by far-red light and paralleled growth in the respective tissues.

Barkley and Evans (1970) reported that auxin inhibited growth of pea (Pisum sativum L.) stems for about 10 minutes followed by a sudden burst of growth for about the next 25 minutes. This response could be inhibited by cycloheximide. This effect was apparently not affected by ethylene as tissues appeared to be insensitive to ethylene over the short time frame in which measurements were taken and there was no increase in endogenous ethylene levels. Hall et al. (1985)

also reported a short term stimulation of growth by auxin and that the degree of stimulation was greater in decapitated plants when auxin was applied to the epidermis than to the cut end. Carrington and Esnard (1988) reported that auxin response was longer lasting when applied basipetally to watermelon (Citrullus vulgaris Schrad. var vulgaris) hypocotyl segments. They reported that auxin stimulated growth in all zones of the excised segments of the hypocotyl, but, only stimulated elongation in the most basal zone and only in intact seedlings. They also pointed out further problems with using excised segments in predicting auxin response in intact seedlings, citing the modifying effects of the apex and cotyledons which would not be taken into account in excised segments.

The opening of the hypocotyl hook to position the above-ground plant parts in an upright position requires a greater elongation rate of the inner side of the hook and occurs in response to red light. In the case of mouse-ear cress (Arabidopsis thaliana L.) hook development may be primarily a physical process requiring the retention of the seed coat. Mutants which do not retain their seed coats during emergence do not develop hypocotyl hooks (Mirza, 1987). The effects of growth regulators on this process have been investigated. Kang and Ray (1969a) reported that hook opening was inhibited by low concentrations of auxin and promoted by gibberellic

acid (GA), Ca^{++}, K^{+}, Co^{++}, and Ni^{++}. High concentrations of auxin caused closure of already opened hooks. They concluded that light acted by some mechanism other than lowering the auxin level in the tissue, and that the process was assisted by GA although endogenous GA levels were not changed. The interaction of ethylene and CO_2 was found to be more important: CO_2 production was stimulated by red light and promoted hook opening while ethylene inhibited opening (Kang and Ray, (1969b). Ethylene production was inhibited by exposure to red light. Furthermore, the effect of auxin in inhibiting hook opening was reported to be due to the stimulation of ethylene production.

Tropic responses are important systems for studying the differential stimulation and inhibition of growth in plant parts. As reported for sunflower (Helianthus annuus L.) hypocotyls grown horizontally, growth is stopped on the upper side while the relative elemental elongation rate (RELEL) is increased on the lower side in the elongating region until the hypocotyl is once again growing upright and RELEL's of both sides become equal (Berg et al., 1986). According to Digby and Firn (1979) geostimulation causes the inner side of the curve to cease elongation while the outer side is accelerated. Autotropic straightening initiated when horizontally grown plants are returned to an upright position occurs by a similar mechanism beginning in the more apical

zones and proceeding basipetally (Firn and Digby, 1979). The magnitude of the rate differential of upper and lower RELEL's determines the rate of bending (Barlow and Rathfelder, (1985). Similar results were reported by Firn et al. (1978) who also stated that the sites of geo-perception and geo-response must be intimately associated and that the magnitude of the response makes it difficult to attribute the changes to lateral redistribution of auxin. Digby and Firn (1979) concluded that the rapid change in differential growth of elongating shoots may be too fast for hormonal control to be involved in initiating gravitropic response. A similar conclusion was reported by Ganot and Reinhold (1970). They reported that acid buffers, but not auxin, could restore gravitropism to etiolated sunflower hypocotyls and that this effect was insensitive to auxin inhibitors. Auxin levels were reported to decrease basipetally in oat (Avena sativa L.) coleoptiles below the region of rapid elongation (Kessler et al., 1985). However, the changes in auxin level reported may be below the point at which a tropic response might be initiated. In a review of tropic responses in plants, Firn and Digby (1980) stated that "the belief that hormones regulate geo-responses persists even though confirming demonstration of appropriate hormone changes has not come forth". In contrast to the results reported above for sunflower, and a few years after the Firn and Digby review,

Bandurski et al. (1984) reported that gravistimulation induces an asymmetric redistribution of auxin in corn mesocotyls. Barlow and Rathfelder (1985) agreed that asymmetric redistribution of auxin to stimulate the more rapid elongation of one side may occur, but it could be a redistribution of an elongation inhibitor away from the more rapid side.

An affect of auxin on differentiation was reported by Morris (1977). Fascicular and interfascicular cambium was less well developed in the nonauxin-fed half of two-branched dwarf pea stems in which the other branch had been supplied labelled auxin. No label activity was reported for the auxin-starved branch. Increasing indole-3-acetic acid (IAA) levels in pea roots were reported to lead to more complex vascular arrangements in pea roots (Torrey, 1957). Sachs (1975) reported on the effect of auxin in differentiation and maintenance of transport channels in beans. Addition of auxin to cut stems increased transport in developing vascular elements. A part of the action of auxin in this system was to inhibit lateral neighbor cells of recently differentiated vascular elements from becoming vascular elements while inducing the cells immediately below to differentiate as new vascular elements.

The site of auxin action in elongating tissues is thought to be in the epidermis (Masuda and Yamamoto, 1972),

and possibly in the central core (Burström et al, 1967). Thimann and Sweeney (1937) reported that auxin increases the rate of protoplasmic streaming in epidermal cells, illustrating that auxins are active in cellular processes. However, it has been reported that protoplasmic streaming is not the mechanism by which auxin promotes cell elongation (Cande et al., 1973). Peeled stem sections of pea are unresponsive to auxin but in an acidified incubation medium they are stimulated to elongate by fusicoccin indicating that peeling the epidermis removes the auxin responsive tissue (Brummell and Hall, 1980). Masuda and Yamamoto (1972) reported that a few layers of the cortical cells adjacent to the epidermis may be necessary for the auxin action on the epidermis to occur.

Cleland and Haughton (1971) reported that auxin reduced the relaxation and relaxation modulus of oat coleoptile sections. These measurements are related to the extensibility of the cell wall. Wall extensibility is necessary for cell expansion, but is not the only important factor (Coartney and Morré, 1980a). Matyssek et al. (1986) reported that water uptake and wall extensibility colimit rate of cell enlargement. Kutschera and Schopfer (1986) reported that IAA-induced changes in extensibility was strictly correlated with growth rate in corn coleoptiles.

The acid growth hypothesis and the involvement of auxin

therein has received a good deal of attention but there are many questions and conflicting reports as to the actual mechanisms of proton extrusion and to its overall importance (Taiz, 1984). Acidification of the incubating medium by auxin only occurred when the medium included calcium or magnesium, but occurred in fusicoccin-treated segments with or without the divalent cations suggesting that cortical cells were responsive to growth stimulation by fusicoccin but not auxin (Brummell and Hall, 1981). Cohen and Nadler (1976) concluded that auxin-induced acidification involved a Ca^{++}-H^{+} exchange. Monovalent cations did not enhance acidification. Calcium did not appreciably reduce auxin-induced proton extrusion, nor reduce capacity of walls to undergo acid-activated loosening in oat coleoptiles (Cleland and Rayle, 1977). In mung bean [Vigna radiata (L.) Wilczek, also known as Phaseolus aureus L.] hypocotyls, fusicoccin induced fresh weight increase and acidification of the medium in all sections. Auxin caused a similar response only in elongating sections (Masuda and Yamamoto, 1972). Proton extrusion induced by fusicoccin reverses the abscisic acid (ABA) delay of germination in Cicer arietinum L. which was attributed to an increase in wall pH (Labrador et al., 1987). Similar results were reported by Kutschera and Schopfer (1986) for corn coleoptile segments. They also reported that ABA inhibited growth stimulation by auxin and acid. Sensitivity

to acid pH and auxin increased basipetally from the hook leading Goldberg and Prat (1981) to conclude that when auxin fails to induce proton extrusion it doesn't induce growth. Vanderhoef et al. (1977) reported on a two-phased stimulation of soybean hypocotyl elongation. The first was a transient increase in elongation rate in response to an acid medium (pH 4) and auxin. Auxin also induced a persistent increased elongation rate when pH was increased to 6. Addition of 1 mM N-2-hydroxyethylpiperazine-N'-2-ethansulfonic acid (pH 7) inhibited the first response to auxin but not the second. This lead to the conclusion that protons are not involved in the longer-term affects of auxin on elongation, but they did not rule out their effect on the initial, transient response. The epidermis, or at least the cuticle had to have been abraded, for acid-induced elongation to be observed. Prat et al. (1984) reported that mung bean hypocotyl elongation was inhibited by Ca^{++} and Mg^{++} but was promoted by H^{+}. Alternating additions of Ca^{++} and H^{+} gave responses characteristic of the last added, although the H^{+} effect was diminished as more Ca^{++} was added.

While acidification may be necessary for wall loosening, auxin may promote growth by affecting other processes in addition to, or instead of acidification (Brummell, 1986). According to Hanson and Trewavas (1982), the stimulation of growth in excised tissues represents largely an accelerated

recovery from injury and that auxin stimulates growth only in cells which are predisposed to grow (predisposition mechanism unknown), i.e., auxin activates preexisting growth potential which was interrupted by excision. A possible effect of auxin in promoting cell expansion may be to increase activity of enzymes involved in breaking of cross-links in the polysaccharide matrix, which would allow cell expansion (Sargent et al., 1973). An effect of auxin to increase water-soluble xyloglucans in cell wall polysaccharides, but not in hemicellulases, was reported to be important in wall loosening via the stimulation of enzymes which depolymerize hemicellulose xyloglucans (Inouhe et al., 1984). Auxin has been reported to stimulate incorporation of ^{14}C-glucose into cell wall fractions in oats, corn, soybean, and pea (Coartney and Morré, 1980b). Galactose inhibited auxin-induced cell elongation by inhibiting the incorporation of glucose into cell wall components of oat coleoptiles (Inouhe et al., 1986), more specifically, the synthesis of UDP-glucose.

Goldberg et al. (1986a) reported on the development of isoperoxidases in mung bean hypocotyls. Peroxidase activity was reported to develop concurrently with decrease in cell wall plasticity. Localization of three peroxidase activity peaks was dependent on stage of cell development; it was highest in the cytoplasm of yonger cells, but in the cell walls and intercellular spaces of older cells. Corresponding

to the peroxidase activity was an increase in pectin content in the middle lamella (Goldberg et al., 1986b). The less soluble pectin fractions increased as cells matured, corresponding to the decrease in wall plasticity. Similar changes in peroxidase activity were reported by Chanda et al. (1986b) in pearl millet (Pennisetum americanum L. Leeke cv. BJ-104). They also reported that cytoplasmic IAA-oxidase was inversely related to elongation and that cytoplasmic and wall bound esterases increased during elongation. Their overall conclusion was that the phenolic content was more important than wall peroxidase in controlling the cessation of elongation (Chanda et al., 1986a). Katsumi (1985) reported that brassinosteroids enhance auxin-induced elongation of cucumber (Cucumis sativus L.) hypocotyls by making the tissue more responsive to auxin, and that their growth promoting effects were independent of gibberellic acid (GA).

Key et al. (1960) reported that auxin (low concentrations of 2,4-dichlorophenoxyacetic acid [2,4-D]) induced growth involved the growth of mitochondria; they specifically noted the increase in acid-soluble nucleotides, phospholipids, and RNA. Inhibitors of RNA and protein synthesis such as chloramphenicol and Actinomycin-D were reported to inhibit IAA-induced growth (Nooden and Thimann, 1963). RNA and protein synthesis were determined to be essential processes for growth induced by low concentrations

of 2,4-D (Key, 1964). The herbicidal action of 2,4-D at high concentrations could be associated with the renewal of RNA and protein synthesis leading to massive tissue proliferation, disorganized growth, and finally death of the tissue (Key et al., 1966). Histologically, this is seen as a distortion of vascular tissue, excessive proliferation of meristematic tissue, and lack of appropriate differentiation (Eames, 1949). Inhibition of growth-dependent protein metabolism was given as a possible explanation of the inhibitory effect of chloramphenicol on auxin-induced cell expansion (Setterfield, 1970) and incorporation of leucine in artichoke tubers (Noodén and Thimann, 1963).

Helgerson et al. (1976) reported that treatment with IAA resulted in an increase in microviscosity of isolated soybean hypocotyl plasmalemmas due to a closer packing arrangement of membrane lipids and a reorientation of large molecules from regions of low to high microviscosity within the membrane. This change in membrane conformation was seen in electron micrographs which showed that IAA-treated membranes were thinner than controls. Localization of auxin receptors on the outer surface of the plasmalemma has been reported (Hertel et al., 1972), as have β-glucan synthases (Haas et al., 1985). Ultrastructural changes in membranes induced by auxin were reported to be reversible by application of 0.5 M $CaCl_2$ by Morré and Bracker (1976). The condition of the

membranes reflected the last treatment of the series when IAA and $CaCl_2$ were alternated. $CaCl_2$ alone increased thickness of membranes by 15 to 20% while IAA alone decreased membrane thickness by 10 to 15%. This can be taken as evidence of a direct response of cell membranes to growth-regulating agents. Transient alterations in membrane structure in cultured cells of thorn apple (Datura inoxia Mill.) leaf explants when sucrose is taken up from the medium can be magnified by kinetin, and to a lesser extent, by auxin (Brossard-Chriqui and Iskander, 1982). The strongest alterations were seen near intercellular spaces and may affect membrane permeability.

Katsumi et al. (1965a) concluded that IAA and GA were both required for normal growth, although their modes of action were different. Holm and Key (1969) reported that GA_3 inhibited IAA-induced ethylene synthesis and stimulated hypocotyl elongation through an additive influence with IAA in 'Hawkeye 63'. However, the cotyledons must be present for GA stimulation to occur (Holm and Key, 1969; Katsumi et al., 1965b). Actinomycin-D and cycloheximide severely inhibited GA-induced growth. ABA had no affect on GA-induced elongation in the elongating region of the hypocotyl but slightly inhibited in the apical region. GA stimulated hypocotyl elongation in three species of crucifers (lettuce, cress, and Hyoscyamus niger) (Frankland and Wareing, 1960).

In pea, the effect of GA on stem elongation was reported to be primarily due to an increase in cell division (Arney and Mancinelli, 1966). The increase in cell division was accomplished by increasing mitotic activity in meristems and causing vacuolated cells to resume cell division. Degani and Atsmon (1970) reported that GA induced elongation and enhanced DNA synthesis in cucumber. Since there was no apparent increase in number of cells, they postulated that increased DNA is primarily a consequence of enhanced growth rather than the cause, but some increase in DNA synthesis could be required for enhanced growth. GA stimulated stem elongation in peanut (Arachis hypogaea L.), though not as much as growth in darkness, and there was a differential response among cultivars (Gardner, 1988). GA stimulates the elongation of epidermal cells in the scutellum of wild oats (Avena fatua L.) (Rao and Raju, 1985). GA-induced increase in internode length may be mediated by enhancement of polyamine synthesis via ADC requiring as well de novo RNA and protein synthesis (Kaur-Sawhney et al., 1986). Polyamines such as putrescine, cadaverine, and agmatine, as well as possible putrescine precursors ornithine and arginine, were reported to enhance GA-induced elongation and to overcome inhibition by arcaine in lettuce (Cho, 1983). Murray and Acton (1974) reported that GAs completely reversed dwarfism in white lupine. Dwarf and semi-dwarf wheat cultivars were

less responsive to GA than standard height cultivars (Allan et al., 1959). However, GA is important in promoting stem elongation in rice (Oryza sativa L.) (Khan et al., 1988; Suge, 1985) and GA concentration increases in submerged floating rice cultivars (Suge, 1985). Sponsel (1986) identified GA_{20} and GA_1 as the important GA's in the elongation of tall ('Alaska') and dwarf ('Progress No. 9') pea. GA_{20} promoted elongation in both cultivars. It was suggested that Progress No. 9 may convert GA_{20} to GA_1 in the dark since GA_{20} metabolism was greater in the dark than in the light. Furthermore, Progress No. 9 may have been unable to hydroxylate GA_{20} under these conditions, although elongation was still enhanced. Red light inhibited elongation in both cultivars, although more dramatically in Progress No. 9. Progress No. 9 is largely insensitive to endogenous GA. This led to the suggestion that the primary effect of red light was on the uptake, compartmentation, or 3β-hydroxylation of GA_{20}.

Cytokinins were reported to inhibit hypocotyl response to auxin, and the effects were dependent on whether auxin or cytokinin was added last (Vanderhoef et al., 1973). The degree of cytokinin effectiveness on auxin-induced elongation depended on which cytokinin was administered: zeatin and isopentenyladenine were more effective then kinetin or benzyladenine. Sustained auxin-induced growth is influenced

by the need for continuing synthesis of new cell wall matrix components (Brummell and Hall, 1985).

Auxin-stimulated growth was reported to be inhibited by catechins in lettuce (Lactuca sativa L.) (Buta and Lusby, 1986). Catechin and epicatechin are thought to act by inhibiting peroxidative oxidation of IAA which would inhibit the continued synthesis and transport of auxin, although this mechanism is not certain. An unidentified inhibitor of auxin stimulation of positive curvature in Avena curvature assay was reported to be correlated with cessation of mesocotyl growth in etiolated oats and inhibition of mesocotyl growth in corn under red light (Kessler et al., 1985). Removal of the endosperm was reported to retard coleoptile growth in oat as this removed the source of sugars, amino acids, and auxins needed to promote growth (Kamisaka et al., 1988).

The biosynthesis of ethylene is important when considering its direct effects on plant tissues as well as its interaction with other compounds. Methionine has been established as the major, if not sole precursor of ethylene in higher plants (Baur and Yang, 1969; Owens et al., 1971). Ethylene only occurs in living plant tissue (Sisler and Yang, 1984). Methionine is converted to ethylene via a pathway leading through S-adenosylmethionine (SAM) (Adams and Yang, 1977), to 1-aminocyclopropane-1-carboxylic acid (ACC) which is the direct precursor of ethylene (Adams and Yang, 1979),

(Konze and Kende, 1979), (Stegink and Siedow, 1986), (Yoshii et al., 1980), (Apelbaum et al., 1981a,b). This pathway is well accepted and its regulation has been studied extensively, but the stimulus or messenger which initiates the synthesis is unknown (Yang and Hoffman, 1984). It has been determined that ethylene ultimately is derived from carbons 3 and 4 of methionine (Hanson and Kende, 1976a). The formation of SAM from methionine requires ATP activation of methionine as demonstrated by the results of Murr and Yang (1975). The reaction was inhibited by known inhibitors of pyridoxal phosphate-mediated reactions and uncouplers.

Boller et al. (1979) developed an assay for the enzyme converting SAM to ACC (ACC synthase) and demonstrated that this was the primary limiting step. The rin mutant (nonripening) of tomato (Lycopersicon esculentum Mill.) contains much lower endogenous levels of this enzyme than wild types and producers considerably less ethylene. A previously suggested pathway involving α-keto-γ-methylthiobutyrate (KMB) (Durham, 1971) is no longer accepted. The site of ethylene formation from ACC was reported to be associated with the vacuoles since up to 90% of the ACC content may be found in vacuoles, and intact tonoplasts are required for ethylene synthesis (Mayne and Kende, 1986). This is in contrast to the previous suggestion by Burg and Thimann (1960) that ethylene production was

associated closely with mitochondria.

IAA has been reported to affect ACC synthase (Yu et al., 1979; Abeles, 1966) but not the conversion of ACC to ethylene (Yu and Yang, 1979). Abeles and Rubenstein (1964) demonstrated the regulation of ethylene evolution by IAA, β-(indole-3)-propionic acid (IPA), γ-(indole-3)-n-butyric acid (IBA), and α-napthyleneacetic acid (NAA). NAA at $5X10^{-4}$ M, for example, stimulated an increase in ethylene synthesis in vegetative tissues of corn, kidney bean (Phaseolus vulgaris L.), tomato, Zebrina pendula Schnizl., Coleus blumei Benth., pea, cassava (Manihot ultissima Pohl.), tobacco (Nicotiana tabacum L.), and coffee (Coffea arabica L.) but inhibited ethylene evolution in ripening tomato, apple (Malus pumula Mill.), and pear (Pyrus communis L.) fruit tissue. Cobalt has been reported to have a stimulatory effect on cucumber hypocotyl elongation by inhibiting ethylene production by Grover and Purves (1976), likely through some interaction with auxin. This would indicate that cobalt indirectly limits ACC level. The role of peroxidative IAA-oxidase in ethylene synthesis was investigated by Fowler (1971). He concluded that either the oxidase is independent of auxin stimulation of ethylene or that ethylene mediates its activity. Continued presence of free auxin is required for ethylene production, which indicates that ACC synthase is highly labile (Kang et al., 1971). Polar auxin transport

(Goldsmith and Ray, 1973) is probably important in maintaining the auxin level and may be regulated by flavonoids (Jacobs and Rubery, 1988). The magnitude of ethylene synthesis may be dependent on auxin concentration (Sakai and Imaseki, 1971). Inhibitors of RNA and protein synthesis were reported to inhibit auxin-stimulated ethylene production in relation to ethylene inhibition of bean hypocotyl hook opening (Kang and Ray, 1969c), and in pea stem elongation, but to a lesser extent in maturing apple fruit (Lieberman and Kunishi, 1975). In mung bean, the region of the hypocotyl just below the hook was most sensitive to auxin induction of ethylene synthesis (Sakai and Imaseki, 1971). Furthermore, the site in bean hypocotyls which evolved more ethylene in response to decapitation, 2,3,5-triiodobenzoic acid (TIBA, an inhibitor of polar auxin transport), photoperiod, or plant orientation (geotropism experiments) also contained higher levels of auxin. However, direct mediation of gravitropism by ethylene is not evident (Harrison and Pickard, 1986).

The conversion of ACC to ethylene by the ethylene-forming enzyme (EFE) can be inactivated by an inhibitor protein isolated from mung bean hypocotyls by Sakai and Imaseki (1973). This protein was reported not to directly affect auxin stimulation of ACC synthase or uptake of ACC (Todaka and Imaseki, 1986). It was reported to bind to the

EFE and also to block the conjugation of free IAA to aspartate, but these were reported to be separate reactions (Sakai and Imaseki, 1973). The tissue specificity of this protein was also reported. It did not inhibit ACC-dependent ethylene production in mung bean hypocotyl segments with the epidermis removed, but did work in peeled segments (Todaka and Imaseki, 1985). This suggests that epidermal cells are not required directly for ethylene synthesis, but may help regulate ethylene synthesis because of the role of epidermal cells in auxin-promoted elongation and auxin stimulation of ACC synthase (Todaka and Imaseki, 1986). The actions mentioned above were found to occur in mung bean and pea, weakly in apple fruit slices, and not at all in wheat coleoptiles. Further work on this protein indicated that the effect of reducing ethylene synthesis was due to a reduction in ATP by the protein, although whether this was due to uncoupling of oxidative phosphorylation or hydrolysis of ATP was not determined (Sakai, 1982). However, the reduction in ATP did not account for all of the inhibition of ethylene synthesis in tissues treated with the isolated protein, ethylene synthesis being more sensitive to the protein than was ATP reduction. Burg and Thimann (1960) had suggested earlier that ethylene production in apple fruit slices required energy at some point.

While auxin levels have been shown to affect ethylene

levels, the reverse may also occur. Lieberman and Knegt (1977) reported that ethylene-treated pea stems contained less than half the IAA found in controls. Chkanikov et al. (1985) reported that exogenously applied ethylene, ACC, or ethrel caused a decrease in free-IAA by intensifying the conjugation of IAA and that IAA oxidase is not as important in controlling IAA level, in contrast to Buta and Lusby (1986). Ethylene also inhibits the movement of auxin in plant tissues (Burg, 1968), both laterally and polarly (Burg and Burg, 1967).

Phytochrome control of ethylene synthesis was reported by Goeschl et al. (1967). Red light markedly inhibited ethylene synthesis in pea epicotyls within 2 hr following exposure. Far-red light was shown to reverse the effect of red light on ethylene production and stimulate growth of epicotyls. According to Vangronsveld et al. (1988) this effect is due to a reduction in free ACC. Malonyl conjugation of ACC was reported in response to red light and was reversible by far-red light.

Fuchs and Lieberman (1968) reported on the interaction of ethylene, IAA, GA, and kinetin on pea, radish, and cucumber seedling growth. Kinetin stimulated ethylene in 3 or 4-day-old seedlings, but not in 6-day-old seedlings, where it appeared to increase the effects of IAA. Kinetin-induced ethylene was inhibited by cycloheximide, cupferon, N-

ethylmaleimide but these substances did not significantly effect the endogenous ethylene-forming system. Ethylene and GA had an antagonistic interaction. Stewart et al. (1974) described the antagonism between GA and ethylene countereffects of secondary reactions in cortical cells from the subhook region of pea. The swelling response to ethylene opposed the elongation promotion by GA, but GA did not influence effects of ethylene in arresting cell division. The GA-insensitive pea phenotype, erectoides, produces 2 to 3 times as much ethylene as dwarf pea. This suggests that ethylene action in erectoides may be related to its lack of response to GA (Ross and Reid, 1986). Addition of ethephon to dwarf peas produced plants which resembled erectoides, both in terms of overall length and in the anatomy of cortex and epidermal cells. The epidermis and outer cortex of typical erectoides plants have a reduced number of cells. The elongation of the cells in these regions of erectoides tends to be reduced when compared to normal pea plants. The outer cortex cells were described as being almost isodiametric. Aminoethoxyvinylglycine (AVG), an inhibitor of ACC synthase, increased internode length in the erectoides pea genotype but had no effect on dwarfs. GA and AVG added together to erectoides had a synergistic effect; internode length was increased to 75% greater than the sum of GA and AVG increases when separate. Sommer (1961) reported that

kinetin, IAA, and sucrose together suppressed elongation and promoted lateral expansion in etiolated pea stems; GA, IAA, and sucrose had the opposite effects. The action of kinetin was inhibited by $CoCl_2$. GA and IAA, alone or with sucrose promoted elongation. Similarly, Lau and Yang (1973) reported that kinetin had a synergistic effect on IAA-induced ethylene production, accomplished by stimulating IAA uptake and suppressing IAA conjugation in mung bean hypocotyls. In potato (Solanum tuberosum L.) tubers; however, ethylene was reported to prevent tuberization and formation of starch storage cells. A possible explanation given was the inhibition of cytokinin function by ethylene (Dimalla and van Staden, 1977). Although soil-applied ethylene increased the volume of sugar beet roots, there was a decrease in sucrose concentration. The overall result was no net increase in sucrose recovery per hectare (Freytag et al., 1980). Dunlap and Morgan (1977) reported that cytokinin, ethylene, and GA reversed dormancy in lettuce, but by different mechanisms. Kinetin inhibited the dormancy-reversing action of ethylene and GA only in the presence of light.

Ethylene has been reported to be involved in several stress-related processes such as abscission, stomatal closure, and control of the opening and closing of flowers (Sisler and Yang, 1984), usually in an interactive system with another hormone type. Rapid production of ethylene in

response to wounding has been reported for a number of species (Saltveit and Dilley, 1978; Hanson and Kende, 1976b; Yu and Yang, 1980). Even in a mouse-ear cress mutant which is insensitive to the hypocotyl elongation and other growth regulating effects of ethylene, wound ethylene is produced at wild-type levels (Bleeker et al., 1988). Although this ethylene production is not generally considered in relation to regulation of development in growing tissues, rates of ethylene evolution tend to be higher in tissues which have been reported to be sensitive to ethylene regulation of growth or otherwise have high ethylene evolution rates, such as etiolated hypocotyls and coleoptiles. Yu and Yang (1980) have reported results of inhibitor studies which indicate that the important step in initiation of wound ethylene is the induction of ACC synthase. Wound ethylene rates tend to be highly variable in woody tissues.

Under anaerobiosis (waterlogged root conditions) ethylene caused epinasty in aboveground parts of tomato (Bradford and Yang, 1980a). The ACC formed in roots in response to low oxygen and transported upward in the xylem and was converted to ethylene in the stems. Continued ethylene production in aboveground parts required a continued flux of ACC. In the tomato mutant, diageotropica, ethylene production was not stimulated by anaerobiosis. Ethylene production in this mutant is less sensitive to auxin

induction than in the wild-type. The limiting step in diageotropica ethylene synthesis is, therefore, the failure to convert SAM to ACC (Bradford and Yang, 1980b). ACC and ethylene formation followed a similar pattern in corn roots (Atwell et al., 1988). The formation of aerenchyma in the roots was influenced by this ethylene interacting with an unidentified (possibly cytokinin) factor from the root apex. Aerenchyma formation occurred in barley (Hordeum vulgare L.) in response to waterlogging but the effect was reported to be due to unidentified factors other than ethylene (Larson et al., 1986). Ethylene stimulates elongation of leaf sheaths and blades of floating rice (Khan et al. 1988) and has a cooperative affect with GA in stems (Suge, 1985). In mesocotyls of japonica rice types, maximum elongation was obtained when ethylene, ABA, and GA were applied together (Takahashi, 1973). Synergistic effects of ethylene were reported for ABA and ethylene but not ethylene and GA. Ethylene effects on leaf growth in rice were mimicked by high CO_2 or low O_2. Leaf growth was inhibited in an atmosphere of ethylene and low O_2, but was stimulated when grown in ethylene plus low O_2 and high CO_2. The interaction of these gases may be more important than light in controlling elongation of rice in waterlogged soils or shallow standing water. The oxygen and carbon dioxide are thought to sensitize seedlings to ethylene (Raskin and Kende, 1983).

Ethylene has been demonstrated to accelerate senescence (Burg, 1968; Sisler and Yang, 1984). In climacteric fruits such as apple (Malus domestica Borhk.) ethylene is produced from ACC by an autocatalytic system in which ethylene stimulates the EFE (Bufler, 1986). As reported by Morin and Hartman (1986), the EFE is responsible for regulating the post-climacteric drop in ethylene production based on results which indicated that free-ACC levels remained high even after ethylene production declined. The EFE can be partially inhibited by carbon dioxide. The initiation of the climacteric is influenced by other hormones. Isopentenyl adenosine (IPA), a cytokinin, reduced ethylene production at all stages of maturation in apple fruit slices and in pre- and post-climacteric tomato and avocado (Persea americana Mill.). IAA suppressed ethylene only in preclimacteric slices. ABA stimulated ethylene in preclimacteric and reduced ethylene synthesis in mid-climacteric stages (Lieberman et al., 1977).

Ethylene may have an indirect role in release from apical dominance. This relationship was reported in Japanese morning glory (Pharbitis nil Chois.) by Prasad and Cline in a series of papers. Ethylene produced in response to shoot inversion restricted growth of the terminal bud before the highest lateral bud could begin to grow out. This effect could be mimicked in upright stems by treatment with ethrel

(Prasad and Cline, 1985a). Mechanical perturbation-induced ethylene released lateral buds from apical dominance by restriction of main stem growth (Prasad and Cline, 1985b). The growing region sensitive to these effects was determined to extend approximately 13 cm from the apex (Prasad and Cline, 1986).

Goeschl et al. (1966) reported that ethylene levels increased in pea epicotyls whose elongation was impeded by glass beads placed over the seedlings. Within six hours after imposition of the stress, ethylene levels increased, and there was a definite curving of the epicotyls. Increasing the depth of the glass beads over the seedlings caused an increase in ethylene levels. In addition, respiration was lower when more beads were added. Impedance due to the beads or treatment with ethylene caused a deceleration of elongation and an increase in diameter of the seedlings. The increased diameter was found to be due to increased cell diameter of the cortical parenchyma cells. The number of cortical parenchyma cells was unchanged.

One of the first effects of ethylene to be described was the inhibition of elongation and increase in radial swelling in legumes (see review of various ethylene effects by Sisler and Yang, 1984). Ethylene has been reported to have an effect on cell wall synthesis in relation to inhibition of elongation and induction of radial swelling in pea epicotyl

(Eisinger, 1983). Inhibition of elongation was reported to occur at ethylene concentrations as low as 10 nl/l (Goeschl and Kays, 1975), whereas radial swelling required higher concentrations in most tissues sensitive to both responses. These results indicate that different mechanisms are being affected by ethylene, either directly or indirectly. Peg development at the hypocotyl-root transition zone in cucumber is inhibited by inhibitors of ethylene synthesis and polar auxin transport (Takahashi and Suge, 1988). Exogenous ACC and ethylene stimulated an overall swelling where the peg normally develops. Exogenous IAA induced a peg-like protuberance. Apelbaum and Burg (1972) reported that auxin-induced ethylene prolonged the cell expansion phase and inhibited elongation growth of the subhook region of pea epicotyls by restricting polar auxin transport. In the apical hook, ethylene and supraoptimal concentrations of 2,4-D inhibited growth by stopping cell division before cells could enter prophase. The stimulatory effect of nonlethal doses of 2,4-D on elongation of corn coleoptile segments was reported to be mediated by 2,4-D stimulation of ethylene synthesis (Zemskaya et al., 1986). This interruption of development probably explains the reduction in seed quality in rose clover treated with nonlethal doses of 2,4-D at the bloom stage reported by Williams and Leonard (1959) by interfering with normal differentiation in the developing

embryos. IAA-induced ethylene has been reported to inhibit growth of buds (Burg and Burg, 1968). Nonpolar transport of applied auxin from leaves via phloem has been reported by Goldsmith et al. (1974) which could influence the auxin supply available to stimulate ethylene synthesis. The combined effects of ethylene on DNA synthesis and microfibril orientation could affect differentiation in pea by prolonging the growth phase of cells, thus inhibiting development of metaxylem and lignification of phloem fibers (Apelbaum et al., 1972; Apelbaum and Burg, 1976). Icekson et al. (1985) have proposed that ethylene inhibits DNA synthesis by lowering polyamine biosynthesis through a reduction in SAM decarboxylase activity.

Ethylene may influence other aspects of cell wall synthesis such as incorporation of proline into structural proteins at the initiation of elongation inhibition/increase in radial expansion and hydroxyproline during extended expansion growth (Burg et al., 1971; Eisinger and Burg, 1972). Ethylene was reported to increase the level of hydroxyproline in pea (Nee et al., 1978). Cleland (1968) concluded that the majority of hydroxyproline proteins in the cytoplasm were not transported to the cell wall, but may be located in enzymatic proteins important to wall development. Of the three fractions of hydroxyproline identified in oat coleoptile, the wall-bound fraction accounted for about 60%

of the hydroxyproline present. This work; however, was done with callus tissues, and Cleland cautioned that these results may not be representative of intact tissues. According to Melan and Cosgrove (1988), ionically bound proteins are not involved in growth of pea epicotyls, but covalently bound proteins may be. It has also been reported the ethylene increases cyanide-resistant respiration in isolated pea mitochondria but does not influence the normal respiratory pathway (Duncan and Spencer, 1987). A morphologically similar response to the elongation inhibition/increased swelling pattern was reported in roots of buckwheat (Fagopyrum sagittatum Gilib.), cucumber, muskmelon (Cucumis melo L. var. melo), corn, and radish (Raphanus sativus L.) in response to treatment with isopropyl N-(3-chlorophenyl) carbamate (Bystrova, 1985).

In ethylene-treated tissues, the innermost layer of microfibrils were oriented longitudinally, which caused cells to expand radially (Eisinger and Burg, 1972; Eisinger, 1971). This effect is primarily important in the outer cortical cells (Taiz 1984). In contrast, in control stems without exogenous ethylene, the innermost microfibril layers were oriented radially, and stem expansion occurred in a more longitudinal direction (Burg et al., 1971; Apelbaum and Burg, 1971). Alterations in microfibril orientation were also noted for IAA and cytokinins. In pea roots it has been shown

that the microfibrils in cell walls in the rapidly elongating region are oriented transversely to the axis (Hogetsu, 1986) changing to a more oblique orientation as cells reach maximum size in the mature zone. Microfibrils were shown to be transverse in elongating cells and oblique in mature cells (Hogetsu and Shibaoka, 1978a). In pea stems, alterations in microfibril pattern were observed 24 h after treatments with ethylene (Henry, 1978). Similarly, Lang et al. (1982) reported that the deposition of microfibrils in pea stems were mostly transverse in controls, but mostly longitudinal in ethylene-treated stems. Furthermore, the change in orientation occurred early enough to be the primary effect of ethylene leading to radial expansion. That these changes occur rapidly and persist after ethylene removal is further evidence that ethylene irreversibly alters microfibril orientation (Nee et al., 1978), but does not affect cellulase activity (Sargent et al., 1973). In several species of green algae microfibril orientation is related to the dynamic shape-changing properties of the cell wall (Haughton and Sellen, 1969). The innermost layer of microfibrils seems to be the most important in controlling the shape of a cell because it is the latest formed, is in closer proximity to the plasmalemma, and has not been loosened by cell expansion as outer layers have been over time (R. Malcolm Brown, Univ. of Texas, Botany Dept., personal communication).

Parallel alignment of microfibrils and microtubules has been shown in some tissues (Hogestu and Oshima, 1986). This was interpreted as an indication that microtubules found near the plasmalemma control the orientation of microfibrils (Cleland, 1971). In cotton fibers, cortical microtubules undergo predictable shifts in orientation during growth (Seagull, 1986). New orientations of wall microfibrils can be predicted by the orientation of the cortical microtubules. The abrupt shifts in orientation of arrays of microfibrils leads to a multilaminate wall composition.

A model for ethylene involvement in this process has been proposed (Eisinger et al., 1983) in pea stems. According to this model, during the first hour after ethylene treatment microtubule realignment occurs, wall physical properties are affected, and a change in direction of expansion begins at about 3 hours. The change in microfibril orientation to a more longitudinal direction along with changes in matrix composition favors lateral expansion.

The mechanism of control of microfibrils by microtubules, however, has been questioned by Preston (1988) because (1) there are examples in plants and algae in which microfibrils are oriented without the involvement of microtubules (Mizuta and Okuda, 1987); Gunning and Hardham, 1982); (2) microfibrils continue in the same orientation when microtubule-inhibiting drugs, such as colchicine, are

administered (Richmond, 1977; Hogetsu and Shibaoka, 1978b); (3) microfibrils and microtubules never come into direct contact with one another because the plasmalemma lies between them. Preston suggests that the orientation of microfibrils is via vector forces acting on the microfibril terminal complexes, causing them to change direction. Such a mechanism, though as yet not experimentally identified, would need to be present even if microtubules do orient microfibrils, because the microtubules must themselves be oriented (Preston, 1988). Although microtubules and microfibrils are not attached, microtubules could still have an effect on the force generation that guides movement of microfibril terminal complexes by creating a shear effect on the plasmalemma (Gunning and Hardham, 1982).

Although ethylene does not interfere with the auxin-induced wall acidification process per se, an acidified cell may be required for ethylene induction of lateral expansion (Taiz et al., 1983). Evidence for this is provided by the ability of neutral buffers to inhibit the ethylene response and by the fact that ethylene reduces the ability of cells to elongate in response to acid pH, causing them to expand laterally instead. The complex interactions between ethylene, auxin, and acidity points out that other factors, in addition to microfibril reorientation and H^+ extrusion, must be involved.

Mita and Katsumi (1986) have reported a possible role of GA in controlling microtubule alignment in a dwarf mutant (d_5) of corn. GA was reported to stimulate transverse orientation of microtubules in cells of the top 1 mm of mesocotyl epidermis which in typical d_5 mesocotyls are longitudinally oriented. The morphological result is longer mesocotyls in GA-treated d_5 plants. This could be related to the recent report by Rood et al. (1988) on the relation of GA concentration to heterosis in corn. Hybrids were reported to contain greater concentrations of GA and to attain greater shoot height than their inbred parents. The inbred shoot height was increased to the level of, or in some cases greater than, that of hybrids in response to treatment with GA.

Temperature-Dependent Hypocotyl Growth Anomaly

Following observation of emergence problems with certain soybean cultivars during the early to mid-1960s Grabe and Metzer (1969) studied the hypocotyl elongation characteristics of 25 cultivars. Several cultivars were reported to exhibit an inhibition of hypocotyl elongation at 25 C. The inhibited hypocotyls often appeared brittle and thickened, and had a tendency to break when grown in deep sand tests. Normal elongation was seen at 20 and 30 C in these same cultivars. On basis of their hypocotyl elongation

characteristics the 25 cultivars were divided into "long hypocotyl", "short hypocotyl", or "intermediate" types. Short hypocotyl types exhibited the elongation inhibition pattern, whereas long hypocotyl types did not. 'Adams', Amsoy, 'Lincoln', 'Shelby', 'Clark', and 'Ford' were identified as short hypocotyl types. Long hypocotyl cultivars were Hawkeye, 'Dunfield', 'Mandarin 507', 'Blackhawk', 'Richland', 'E.M. Manchu', 'B.H. Manchu', 'Grant', 'Muckden', 'Mandarin', Wayne, 'Seneca', 'Wisconsin March 606', and 'Harosoy'. 'Traverse', 'Lindarin', 'Illini', 'Renville', and 'Chippewa' were considered intermediate types.

Several cultivars were subjected to emergence tests from 10-cm deep sand with temperature as the main variable (Gilman et al., 1973). In one set of experiments seedlings were transferred from 20, 25 or 30 C temperature regimes at daily intervals. It was reported that the degree of sensitivity of short hypocotyl cultivars to elongation inhibition was related to the duration of exposure to the 25 C treatment, i.e., as exposure time to 25 C was increased, the inhibition was more severe. The long hypocotyl cultivar Hawkeye was not sensitive to 25 C. Short hypocotyl seedlings transferred from 30 to 25 were not inhibited, suggesting that they had reached some physiologically critical point before the transfer. Hypocotyl length of seedlings transferred from

20 C to a higher temperature was related to the length of time spent at 20 C. Differences in hypocotyl length between long and short hypocotyl cultivars under constant 25 C were not apparent at day three, but were maximally expressed by day 10. In another set of experiments reported in the same paper, in which seedlings were grown at constant temperatures of 1 C increments from 20 to 32 C, hypocotyl length of short hypocotyl cultivars decreased as temperature was increased from 20 to 25 C and increased from 25 to 29 C. Amsoy was the most sensitive of the four short hypocotyl cultivars tested. Laboratory differences between short and intermediate hypocotyl cultivars were not reflected in field emergence experiments (Fehr et al., 1973); however, differences between short and long hypocotyl cultivars were useful in screening against short hypocotyl types.

The bimodal characteristic of the 25 C inhibition was demonstrated in Clark by Samimy and LaMotte (1978), who also reported that sensitivity persists after removal from 25 C and that the period of sensitivity was limited to about six days after planting.

In Clark and Shelby, inhibited hypocotyl elongation was accompanied by increase in size and number of lateral roots (Samimy and LaMotte, 1976), which indicated that the inhibited elongation was the result of a diversion of dry matter to the roots instead of to the hypocotyl. Similar

amounts of dry matter were translocated from the cotyledons in short and long hypocotyl types; however, in long hypocotyl types a greater percentage goes to the hypocotyl than in the short hypocotyl types (Samimy, 1970). Excision of up to 50% of the cotyledon tissue partially reversed the temperature-induced hypocotyl elongation inhibition in Amsoy and Beeson, both short hypocotyl types (Burris and Knittle, 1975). Effect of partial cotyledon removal was greater in Amsoy which is more strongly inhibited at 25 C. Hypocotyl dry weight was reduced by cotyledon excision in long as well as short hypocotyl cultivars; however, since there was only a partial reversal of the temperature-induced elongation inhibition it was concluded that the cotyledons were the production site of an inhibitor (Knittle, 1977).

Genetic control

Grabe and Metzer (1969) concluded that the 25 C inhibition characteristic was genetically controlled and suggested that it involved more than one gene. Clark, Ford, Shelby, Chippewa, and Renville were derived from the three-way cross of Lincoln X (Lincoln X Richland). The fact that some of the cultivars derived from this cross were identified as short hypocotyltypes, and some others intermediate types provides evidence for multiple gene involvement.

Fehr (1973) attempted to select for hypocotyl elongation

from various crosses involving 11 soybean cultivars (4 long hypocotyl cultivars, 3 intermediate, and 4 short). Progeny were grown in 10 cm sand at 25 C. F_1 progeny of long X long, long X intermediate, and long X short crosses exceeded the midparent value for hypocotyl length or were not significantly shorter than the long parent. Segregation from long X intermediate and long X short crosses were typical of a major gene effect, and suggested that the long hypocotyl condition was dominant. Long X long crosses showed limited differences in the F_2 indicating the possibility of genes with small effects. It seems that the long hypocotyl parents differed from the intermediate and short parents by at least one major gene and that variation within types was due to the genes with smaller effects. Hypocotyl length in the F_2 generation of intermediate X intermediate and intermediate X short crosses were similar to those of the intermediate parent. Progeny of short X short crosses were predominantly short, with some apparently intermediate. It was concluded that selection of the long phenotype would exclude most of the short lines but would not be effective in selecting against short hypocotyl lines within a large portion of the intermediate types because progeny of crosses of long x short or long x intermediate lines that are phenotypically long would be heterozygous.

The role of plant growth regulators

Samimy and LaMotte (1976) reported on the effects of various exogenously applied and endogenously measured plant hormones on the temperature-dependent short hypocotyl growth pattern. The inhibition of hypocotyl elongation in Clark grown at 25 c was accompanied by an increase in ethylene evolution. The symptoms were enhanced by application of exogenous ethylene, auxin, and kinetin. The inhibition of hypocotyl elongation exhibited by Clark grown at 25 C could be mimicked in Mandarin and Hawkeye by application of exogenous ethylene. The effect was partially reversed by CO_2. Gibberellin (GA_3) stimulated hypocotyl elongation and partially reversed the 25 C effect in Clark. Another short hypocotyl cultivar, Shelby, was reported to behave in a fashion similar to Clark. Cobalt ion was reported to partially reverse the effect of ethylene in Clark (Samimy, 1978a), increase elongation, and reduce swelling when applied at concentrations up to 1 mM. Cobalt inhibited elongation at 10 mM. PCIB (p-chlorophenoxy-isobutyric acid), an antiauxin, inhibited hypocotyl and primary root elongation (Samimy, 1970).

Keys (1979) reported that although there was an inhibition in length in Amsoy at 25 C, fresh weight was increased compared to 20 or 30 C. There were greater rates of ethylene evolution from the epicotyls, but not from the

cotyledons or hypocotyl. The presence of the cotyledons affected the ethylene evolution of the epicotyl, but not of the hypocotyl. The duration of high ethylene evolution lasted longer in Amsoy at 25 C than in the long hypocotyl cultivar Corsoy.

Knittle and Burris (1979a) reported that variation in hypocotyl length in the field coincided with the 25 C response. The short hypocotyl cultivars used in the study emerged more poorly than the long hypocotyl cultivars. Hypocotyl swelling, however, was not as closely aligned with the long versus short classification. Amsoy 71 had consistently higher values for Hypocotyl Swelling Index (Knittle and Burris, 1979b) than Wayne.

In contrast to Keys (1979), Seyedin (1981) reported that increased ethylene evolution at 25 C was localized in the hypocotyl. Ethylene levels in Amsoy 71 and 'Cutler 71' hypocotyls were significantly higher at 25 than at 20 or 30 C (Seyedin et al., 1982b). There was no difference in ethylene level in Corsoy hypocotyls at the same three temperatures. Cotyledon removal or use of small seed of Amsoy 71 reduced ethylene evolution by the hypocotyls, providing further evidence that the cotyledons may supply a factor important to the regulation of ethylene production. The simultaneous addition of IAA and methionine stimulated ethylene production as did the addition of ACC at 25 or 30 C. These results

suggest that enhanced ethylene production at 25 C required enhanced levels of methionine and IAA. The free-IAA level was found to be higher in epicotyls of Amsoy 71 at 25 C but not in the hypocotyl or cotyledons.

Further evidence for the involvement of ethylene in the short hypocotyl growth pattern is the effect of light on the process. Samimy (1970) demonstrated that exposure to red light would increase hypocotyl length and decrease lateral expansion. Seyedin et al. (1982a) reported similar results and also reported that breakage was not seen in seedlings exposed to light. Far-red light was shown to reverse the effect of red light on soybean hypocotyls (Samimy, 1978b). Morris (1974) reported similar results as to the reversible effects of red and far-red light and proposed that phytochrome was a mediator of the ethylene response.

Lersten and Carlson (1987) provide a general view of soybean hypocotyl anatomy; however, the anatomical features associated with the temperature-induced "short hypocotyl" growth pattern in certain soybean cultivars have not been characterized. Information discussed previously on the effects of some of the environmental and endogenous plant factors on the anatomy of hypocotyls and other seedling organs in other species are useful in consideration of this problem.

MATERIALS AND METHODS

Plant Material

Cultivar selection

Two 'short hypocotyl' and two 'long hypocotyl' cultivars (Grabe and Metzer, 1969) were selected for the study of the anatomy of development in hypocotyls in connection with the temperature-dependent inhibition of elongation. 'Amsoy 71' (Probst et al., 1972) and 'Beeson 80' (Wilcox et al., 1980) were selected as the short hypocotyl types on the basis of poor emergence scores in yield trials (Iowa Agricultural Extension Service, 1984). 'Corsoy 79' (Release notice obtained from G. E. Pepper, Dept. of Agronomy, Univ. of Illinois at Urbana-Champaign), and 'Oakland' (Bahrenfus and Fehr, 1980) were selected as the long hypocotyl types because of excellent emergence ratings in similar tests. In addition, Amsoy 71 and Corsoy 79 had been used in recent research on the effects of ethylene and auxin on the temperature-dependent hypocotyl growth anomaly (Seyedin et al., 1982). Amsoy 71 has been shown to exhibit the hypocotyl anomaly at 25C, while Corsoy 79 does not.

Seed lots

Certified seed of Amsoy 71, Beeson 80, Corsoy 79 were obtained from Sansgaard Seed Farm, Story City, Iowa.

Certified seed of Oakland were obtained from the Committee for Agricultural Development, Ames, Iowa. All seed lots were produced during the 1983 growing season, were 99% pure, and had germination percentages of at least 90% as determined by standard germination tests (Appendix Table A1). Germination was tested periodically during the course of the experiments and was found to remain high. Seed lots were held in cold storage until needed.

Seedling production

To produce seedlings for study, soybean seed of the four cultivars were surface sterilized in 0.07 M sodium hypochlorite (ClNaO) for 30 sec followed by a rinse in distilled water, then 30 sec in 0.1 M hydrochloric acid (HCl) (Seyedin, 1981). Seed were then imbibed for approximately 1.5 hr in running distilled water to remove the surface sterilization solutions and to allow identification of physically damaged seed which were discarded.

Twenty-five seed were placed on moistened seed germination towels (Anchor Paper Co., St. Paul, MN) 10 cm from the top edge of the towel. Seed were oriented with micropyle end down. There were two towels underneath the seed with a third towel placed over the seed. Towels were then rolled with the seed inside and placed in plastic buckets with supports so the towels would remain upright.

The buckets were covered with a clear plastic bag held in place with a rubber band around the top of the bucket (Burris and Fehr, 1971). The buckets were placed in growth chambers (Percival, Boone, Iowa) at 20, 25, or 30 C in darkness. Growth chambers were set at the desired temperature 1 to 2 days prior to the start of the experiment to allow time for the temperature to stabilize. Temperatures in the growth chambers were monitored with a thermometer inside the growth chamber and a built-in 24-hr recording device. Any time the temperature was found to deviate more than about 3 degrees from the desired temperature the seedlings produced were not used. Seed which appeared to have fungal or bacterial contamination were removed from the towels and discarded. Once the seed had been placed in the growth chambers all manipulations were carried out in complete darkness or under dim green safe-lights (Samimy, 1970) constructed from incandescent lamps covered with blue and red filters.

Hypocotyl length and weight measurement

Hypocotyl length (HL) of 25 seedlings was measured in mm with a metric ruler each day for seven days. Hypocotyl length was considered to be the distance from the base of the cotyledons to the hypocotyl-root junction (Figure 1). The same 25 seedlings were used each day. Rate of hypocotyl elongation per day was calculated by dividing the length by

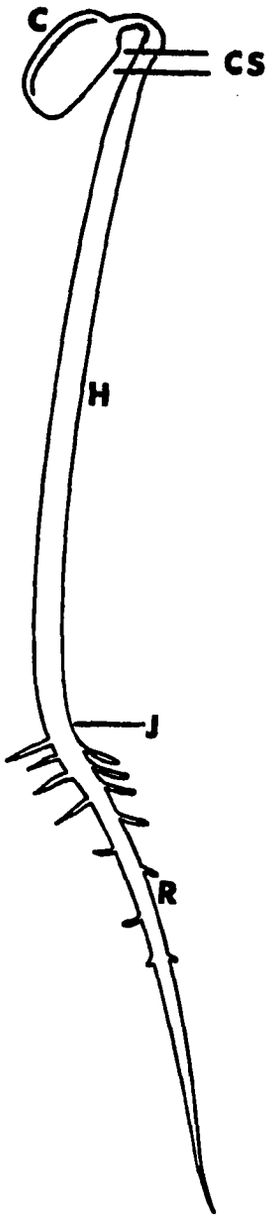


Figure 1. A typical soybean hypocotyl showing region from which cross-sections for anatomical observations were obtained (CS)

C=cotyledons, H=hypocotyl, J=hypocotyl-root junction, R=root

the number of days of growth.

Ten hypocotyls were taken from towels each day and cotyledons and roots were removed. Lengths, fresh weights, and oven-dry weights were determined using these hypocotyls. Fresh (FW) and dry weights (DW) were recorded as the total weight of the 10 hypocotyls. Hypocotyl Swelling Index (HSI) was calculated in the manner of Knittle (1977): HSI=Fresh Weight (mg) per cm). Hypocotyl Diameter (HD) was calculated as $HD=\sqrt{(1/pi)(1cm^3/1000mg)(HSI)}(10mm/1cm)(2)$.

Light Microscopy

Specimen collection, fixation, and sectioning

Specimens for microscopic examination were collected at the time when towels were opened to make length and weight measurements on day two through day seven. Sections approximately 2 cm long were cut from the hypocotyl region immediately below the hook (Figure 1), fixed in formalin-acetic acid (FAA) (Berlyn and Miksche, 1976), and stored in the fixative pending further processing.

Specimens to be cross-sectioned were removed from the fixative and placed in 70% ethanol as a washing step. Five hypocotyl segments collected as described in the preceding paragraph from each temperature-cultivar-day combination were used. Free-hand cross-sections were made with a razor blade and collected in a petri dish containing 70% ethanol.

Sections which appeared to be whole, thin, and flat were placed on a microscope slide, stained with fast green in 70% ethanol, and covered with a cover slip.

Observation of hypocotyl anatomy

Sections were examined using a Bausch & Lomb Balplan light microscope using a 10X objective. Anatomical measurements were made using an eyepiece micrometer. The microscope was calibrated so that micrometer marks could be converted to mm or um units for interpretation. Three cross-sections from each hypocotyl were examined as follows.

Section diameters were recorded as distance in micrometer marks along a transect through the middle of the section from epidermis to epidermis (Figure 2). The diameter of the vascular cylinder (outer edge to outer edge of vascular tissue) was recorded similarly. Within the diameter of the section, the width of the cortex from the edge of the section to the outer edge of the vascular cylinder was measured and the number of cells included in this distance counted. A similar measurement was taken on the opposite side of the section. The diameter of the pith region was measured and the number of cells counted between the inside edges of the vascular tissue. Cortex and pith cell diameters were calculated by dividing the width (in μm) by the number of cells included in each width. A second set of

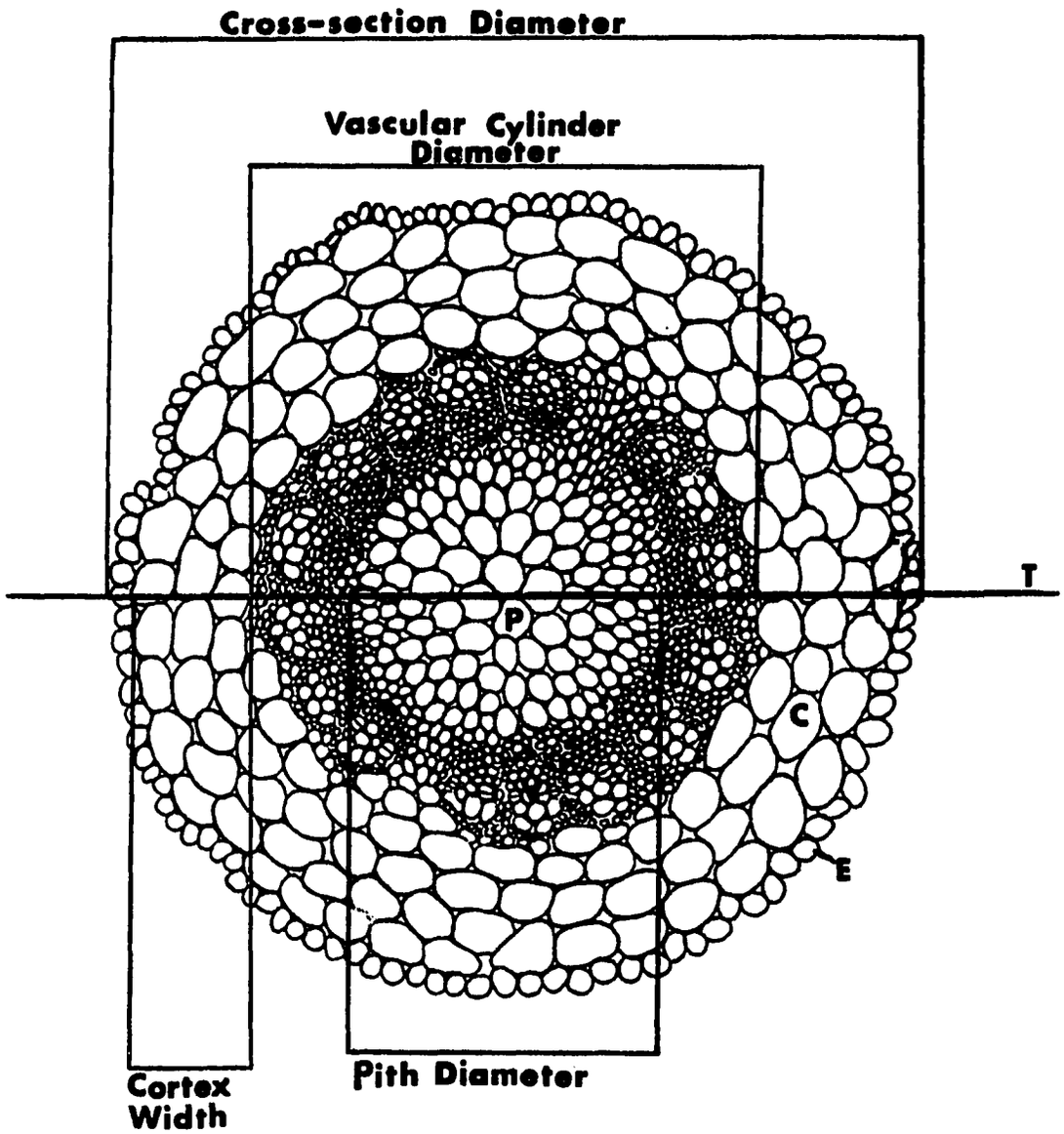


Figure 2. Diagram of a cross-section through a soybean hypocotyl illustrating location of cross-section, vascular cylinder, and pith diameters and cortex width measurements along a transect through the middle of the section (T)

C=cortex, E=epidermis, P=pith, V=vascular tissue

width/diameter measurements and cell counts were made as described above along a transect perpendicular to the first.

Analysis of variance for each parameter was done using either the mainframe or personal computer version of SAS (SAS Institute, Cary, NC). Where there was a significant day component in the overall analysis of a particular parameter, a second set of analyses of variance were run for each day. LSDs for separation of means were calculated using procedures adapted from Gomez and Gomez (1984).

RESULTS

Hypocotyl Morphology

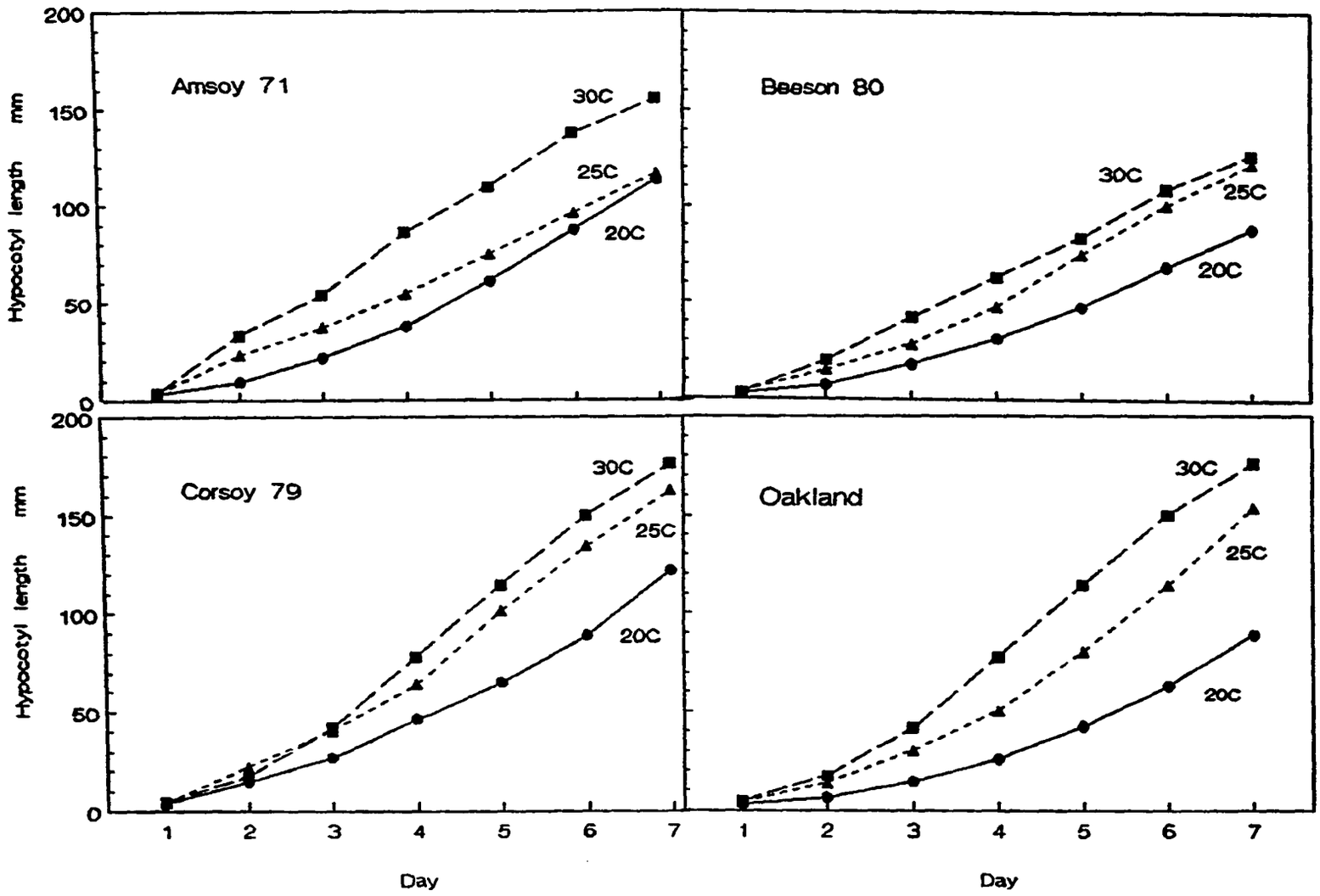
Hypocotyl length

Hypocotyl length, measured daily on seedlings of the four cultivars grown at 20, 25 or 30 C for seven days, are presented in Figure 3. The analysis of variance of hypocotyl length is summarized in Appendix Tables A2 and A3. Cultivar was significant for hypocotyl length at each day as was the temperature x cultivar interaction. Temperature was significant at all days except days two and three. For the purposes of the present study, the interaction is the most important factor, since the primary interest is the pattern of response of each cultivar to temperature.

Hypocotyl length of Amsoy 71 at 20 and 25 C were not significantly different from each other at any day. Length at 30 C was significantly greater than at 20 C at days two through five, and significantly greater than at 25 C at day three. The inhibition of Amsoy 71 hypocotyl length was not as severe as expected on the basis of previous studies on the temperature-dependent growth anomaly in soybean hypocotyls (Samimy, 1970; Seyedin, 1981). Hypocotyl length of Amsoy 71 at 25 was never less than that at 20 C; however, the 25 C curve approaches that of 20 C progressively from day two to day seven. This trend can also be seen when the rates of

Figure 3. Time course of hypocotyl length of Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean grown at 20, 25, or 30 C

Standard errors (cm) are: Day 1=1.1, Day 2=5.6, Day 3=9.9, Day 4=16.07, Day 5=22.0, Day 6=25.6, Day 7=27.52


Amsoy 71
Beeson 80
Corsoy 79
Oakland
30C
25C
20C
Hypocotyl length mm
Day
0
50
100
150
200
1
2
3
4
5
6
7

hypocotyl elongation are compared (Table 1).

Beeson 80 appears to exhibit some inhibition at 25 C at about days three and four, but the inhibition seems to diminish later. Furthermore, hypocotyl lengths at 25 and 30 C were not significantly different over the course of the seven days. As with Amsoy 71, inhibition was not as severe as expected. Length at 20 C was significantly lower than at 30 C at days two and three, but not significantly different from that at 25 C. Beeson 80 had the shortest hypocotyls and slowest rates of elongation of any of the four cultivars at all three temperatures.

Corsoy 79 hypocotyl lengths were not significantly different among the three temperatures, except at day two when 30 C hypocotyl length was significantly greater than 20 C. Corsoy 79 was not expected, and did not, appear to be inhibited at 25 C. In fact, the elongation rate at 25 C is very near that at 30 C.

Oakland responded to temperature in the most linear fashion of any cultivar. This is demonstrated by the progressive spread over time among the three temperature curves, and the fact that rates of elongation increase by about 50% with 5 C increments in temperature. This was expected as Oakland had been selected as a long hypocotyl type. Length at 30 C was never significantly different from 25 C but was significantly greater than 20 C at days three,

Table 1. Rate of hypocotyl elongation of Amsoy 71, Beeson 80, Corsoy 79 and Oakland soybean at 20, 25 and 30 C for seven days

Cultivar	Temperature	Rate of Elongation
Amsoy 71	20 C	12.5cm/dy[a]
	25	13.8
	30	19.7
Beeson 80	20	9.8
	25	14.4
	30	15.6
Corsoy 79	20	12.7
	25	19.2
	30	19.4
Oakland	20	9.1
	25	16.3
	30	21.5

[a]Rate of elongation determined by dividing the hypocotyl length measured on day seven by seven.

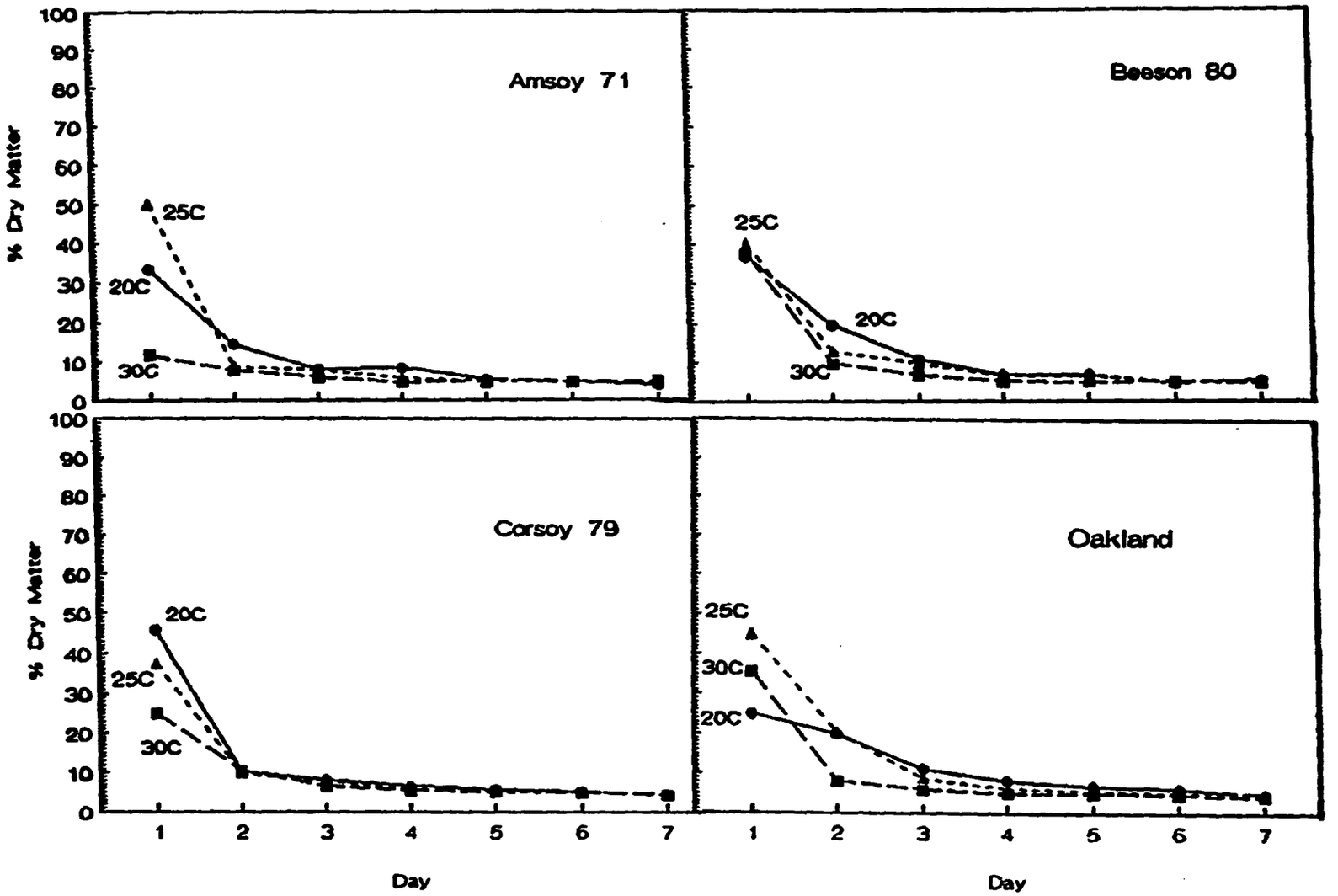
four, five,and seven.

Percent dry matter

The pattern of change in percent dry matter of the hypocotyls over the seven day period among temperatures is similar within each of the four cultivars (Figure 4). The significant day component in the Analysis of Variance is probably due to the rapid drop from day one to day three (Appendix Tables A4 and A5). This reflects the change of the tissue from a germination mode in which most of the

Figure 4. Time course of percent dry matter of Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean hypocotyls grown at 20, 25, or 30 C

Standard errors (%) are: Day 1=12.19, Day 2=1.85, Day 3=0.64, Day 4=1.15, Day 5=0.62, Day 6=0.32, Day 7=0.17


Amsoy 71
Beeson 80
Corsoy 79
Oakland
% Dry Matter
Day
25C
20C
30C
0
10
20
30
40
50
60
70
80
90
100
1
2
3
4
5
6
7

elongation is occurring in the radicle rather than true elongation growth of the hypocotyl. The pattern of dry matter change was consistent among cultivars. Actual percentages differed slightly among cultivars. Differences in percent dry matter among temperatures in all cultivars probably reflects differential rates of imbibition and initial germination due to temperature.

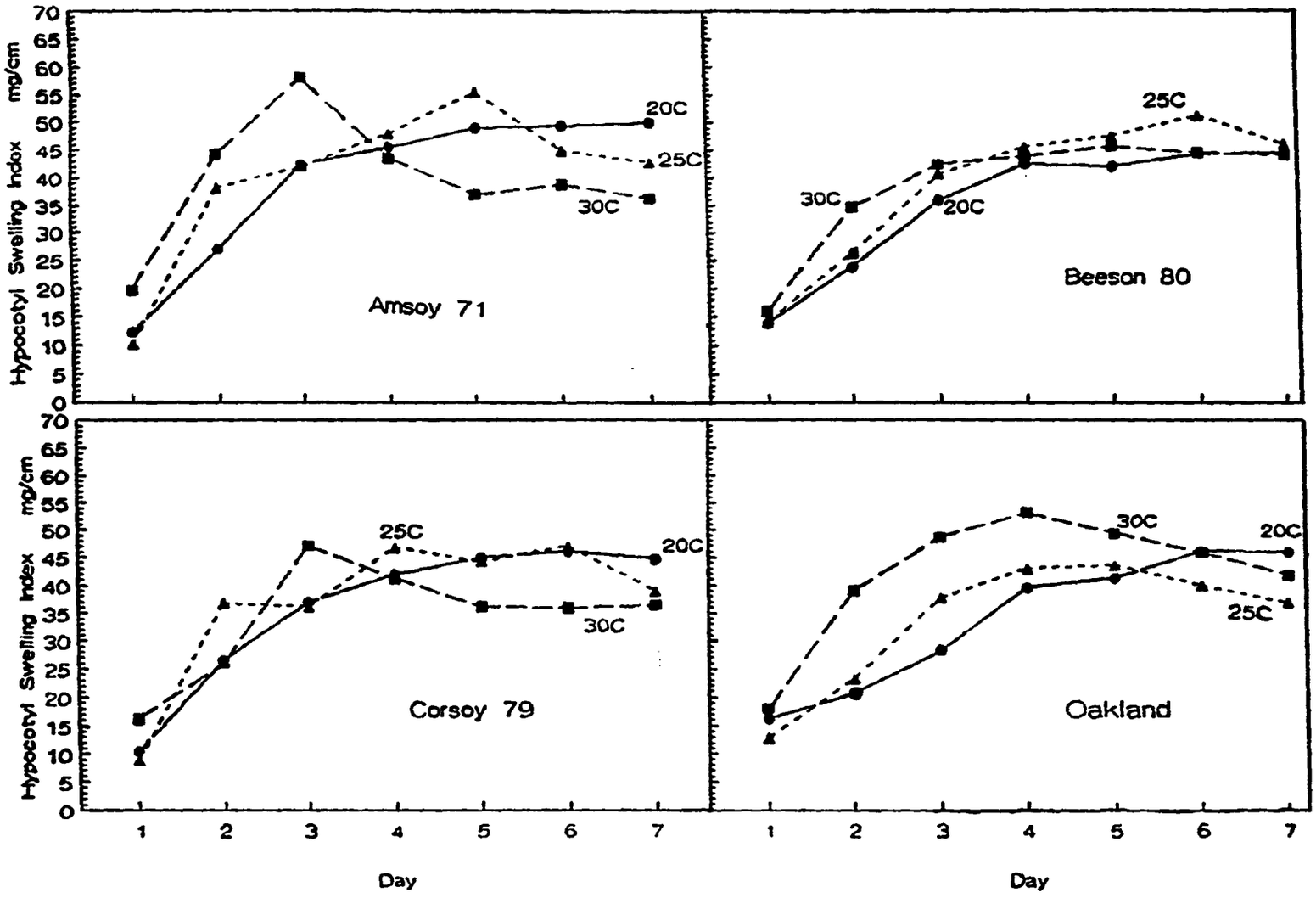
Hypocotyl swelling index

Hypocotyl swelling index (HSI) was calculated in the manner of Knittle (1977). The analysis of variance for HSI is summarized in Appendix Tables A4 and A6. Day and temperature x day were significant at the 0.01 confidence level, and the temperature x cultivar x day interaction was significant at 0.05. Temperature was significant at the 0.01 level at day one, and significant at the 0.05 level at days four and seven. Temperature x cultivar was significant (0.05) at days four and seven.

A possible relation between HSI and hypocotyl length may be seen between days three and four in Amsoy 71 (Figure 5). HSI of Amsoy 71 at 30 C drops sharply from day three to day four, while that of Amsoy 71 at 25 C increases from day three to day five. This is approximately the same time at which the hypocotyl length curves for Amsoy 71 at these two temperatures have their widest separation (Figure 3). In

Figure 5. Time course of hypocotyl swelling index of Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean at 20, 25, or 30 C

Standard errors (mg/cm) are: Day 1=2.38, Day 2=3.32, Day 3=5.33, Day 4=2.88, Day 5=5.67, Day 6=4.06, Day 7=2.22


Hypocotyl Swelling Index mg/cm
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
20C
25C
30C
Amsoy 71
Beeson 80
Corsoy 79
Oakland
1
2
3
4
5
6
7
Day

Beeson 80, HSI increases with time at all three temperatures through day seven.

In both Corsoy 79 and Oakland, HSI at all three temperatures increases until day three or four. After that time HSI at 20 C approaches a plateau, while at 25 and 30 it begins a gradual decline which continues through day seven. The time course of this response corresponds with the slow early rate of increase in length, and the later, more rapid elongation of these cultivars. This time relation is more evident in Oakland than in Corsoy 79.

Hypocotyl diameter by calculation

Hypocotyl diameter was calculated from HSI (Knittle, 1977) so that this method of determining tissue diameter could be compared with direct measurement of hypocotyl cross-sections. Analysis of variance indicated that cultivar, temperature x cultivar, days, and days x temperature were significant at the 0.01 level, while cultivar x days and the three-way interaction were significant at 0.05 (Appendix Table A4). On a per day basis, temperature was significant at days one and seven; cultivar at days two, three, and seven; and the temperature x cultivar interaction at days two, four, and seven (Appendix Table A7).

The general patterns reported above for HSI are consistent with calculated hypocotyl diameter as are the

relationships of hypocotyl diameter with length (Figure 6). This is as expected since HSI is the basis for calculating hypocotyl diameter by the method used.

Anatomical Measurements

Measurements of tissue region diameter, cell number, and cell size were made on cross-sections of hypocotyl material collected from seedlings of the four cultivars grown in rolled towels at each of 20, 25, or 30 C at two, three, four, five, six, and seven days after planting. The measurements reflect the changes which occur in the growing region of the hypocotyl in response to temperature over time, as well as the relationship between the various regions visible in cross-section as contributing to the overall diameter of the hypocotyl. Tables 2, 3, 4, 5, 6, and 7 contain data collected from these measurements at days two, three, four, five, six, and seven, respectively; they also include the Least Significant Difference (LSD) values used to determine statistical significance among temperature treatments within the same cultivar, and among cultivars at a common temperature. The tables are useful for observing the relationship between various anatomical measurements. In addition, Figures 3 through 14 illustrate the changes which occurred in the various parameters measured over time.

Figure 6. Time course of calculated hypocotyl diameter of Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean grown at 20, 25, or 30 C

Standard errors (mm) are: Day 1=0.10, Day 2=0.10, Day 3=0.13, Day 4=0.08, Day 5=0.14, Day 6=0.10, Day 7=0.06

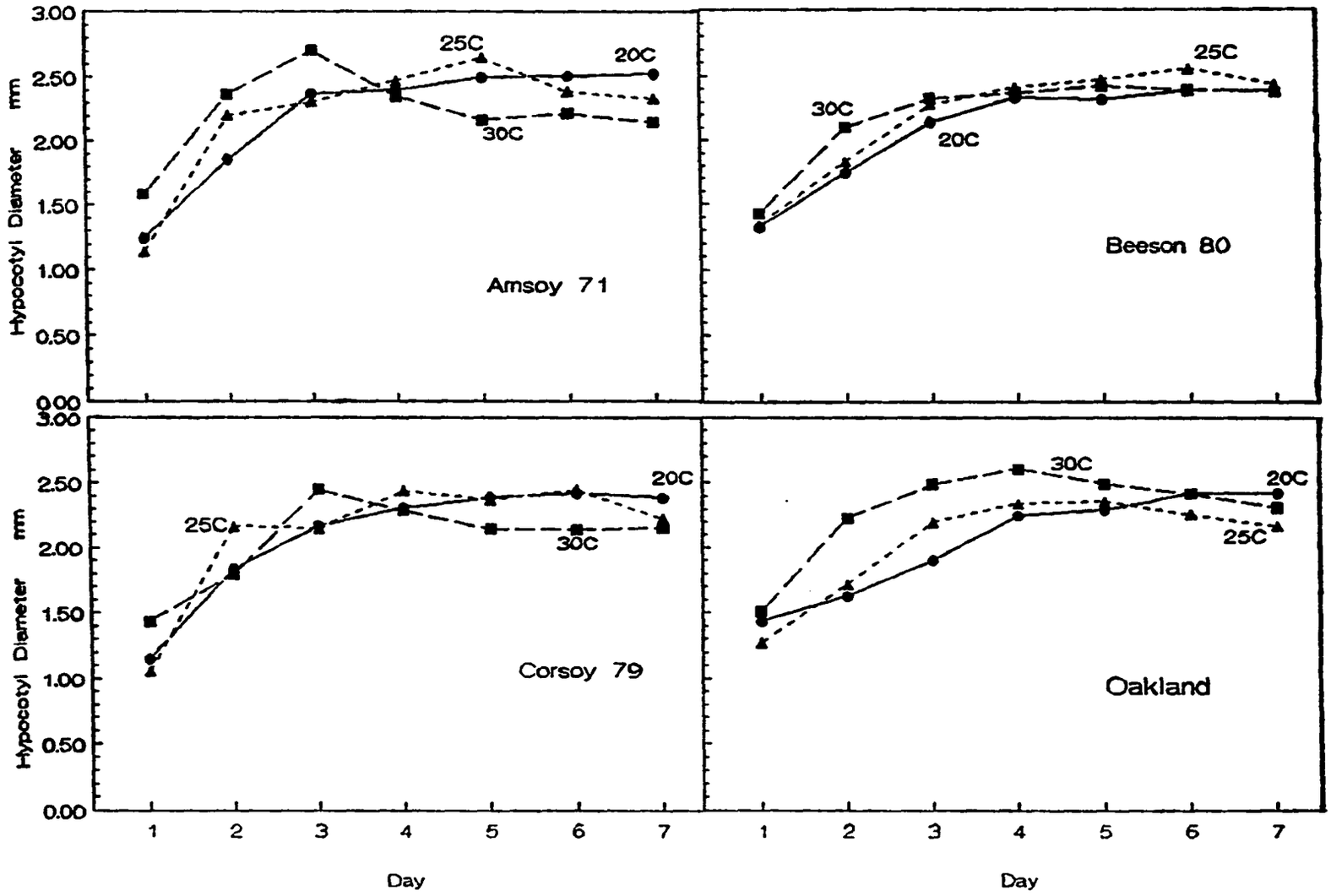

Hypocotyl Diameter mm
3.00
2.50
2.00
1.50
1.00
0.50
0.00
25C
20C
30C
Amsoy 71
Beeson 80
Corsoy 79
Oakland
1
2
3
4
5
6
7
Day

Table 2. Anatomical measurements from cross-sections of 2-day-old hypocotyls of Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean grown at 20, 25 or 30 C

				Pith			Cortex		
Cultivar	Temp.	Diam.	V. C. Diam.	Diam.	Cell No.	Cell size	Width	Cell No.	Cell size
Amsoy 71	20C	2.2mm	0.8mm	0.61mm	12.1	50.3μm	0.68mm	13.1	52.0μm
	25	2.1	0.8	0.85	12.7	47.9	0.64	13.7	46.5
	30	2.3	1.1	0.82	13.7	60.7	0.63	12.2	51.5
Beeson 80	20	2.1	0.7	0.46	10.5	43.9	0.67	13.3	50.3
	25	2.3	0.9	0.63	12.2	52.7	0.66	13.8	47.7
	30	2.3	1.0	0.75	14.0	54.1	0.62	13.5	45.9
Corsoy 79	20	2.1	0.8	0.57	12.2	46.8	0.63	13.0	49.0
	25	2.4	1.1	0.74	12.9	57.6	0.63	13.1	48.0
	30	2.1	1.0	0.76	14.4	53.1	0.54	13.0	41.7
Oakland	20	2.4	0.7	0.45	12.5	37.4	0.60	14.2	42.1
	25	2.3	1.0	0.75	13.9	54.1	0.65	15.0	43.2
	30	2.4	1.1	0.84	14.7	57.7	0.60	13.3	45.5
LSD[a]		-	0.25	0.127	2.54	11.39	0.133	1.58	8.08
LSD[b]		-	0.14	0.108	2.24	7.90	0.098	1.51	5.88

[a]0.05 confidence level, to be used for comparing differences between temperatures within the same cultivar.

[b]0.05 confidence level, to be used for comparing differences between cultivars at the same temperature.

Table 3. Anatomical measurements from cross-sections of 3-day-old hypocotyls of Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean grown at 20, 25, or 30 C

				Pith			Cortex		
Cultivar	Temp.	Diam.	V. C. Diam.	Diam.	Cell No.	Cell size	Width	Cell No.	Cell size
Amsoy 71	20C	2.3mm	1.0mm	0.78mm	14.7	53.5μm	0.65mm	13.5	48.3μm
	25	2.6	1.1	0.85	14.4	59.5	0.76	13.6	55.9
	30	2.3	1.3	0.96	16.3	59.2	0.49	10.5	46.9
Beeson 80	20	2.3	0.9	0.66	12.6	52.6	0.67	14.1	47.5
	25	2.3	1.0	0.75	13.5	55.7	0.62	13.6	45.7
	30	2.1	1.1	0.77	14.7	52.5	0.45	11.3	40.1
Corsoy 79	20	2.3	1.0	0.77	14.5	53.7	0.62	13.0	47.7
	25	2.2	1.1	0.83	14.1	59.0	0.54	11.9	45.5
	30	2.0	1.1	0.80	15.5	51.8	0.44	10.8	40.8
Oakland	20	2.2	0.8	0.58	12.5	46.5	0.66	14.0	47.2
	25	2.5	1.3	1.00	15.4	65.6	0.57	11.7	49.1
	30	2.1	1.2	0.90	15.7	57.5	0.44	11.1	39.7
LSD[a]		0.45	0.29	0.195	3.00	11.53	0.153	1.71	10.45
LSD[b]		0.26	0.14	0.167	1.98	9.60	0.093	1.40	5.98

[a]0.05 confidence level, to be used for comparing differences between temperatures within the same cultivar.

[b]0.05 confidence level, to be used for comparing differences between cultivars at the same temperature.

Table 4. Anatomical measurements from cross-sections of 4-day-old hypocotyls of Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean grown at 20, 25, or 30 C

				Pith			Cortex		
Cultivar	Temp.	Diam.	V. C. Diam.	Diam.	Cell No.	Cell size	Width	Cell No.	Cell size
Amsoy 71	20C	2.4mm	1.2mm	0.87mm	15.1	58.0μm	0.63mm	13.2	48.2μm
	25	2.7	1.2	0.89	15.0	60.0	0.74	12.8	58.1
	30	2.3	1.3	0.97	17.4	60.4	0.46	10.6	45.0
Beeson 80	20	2.4	1.0	0.75	13.4	56.0	0.69	13.7	50.5
	25	2.4	1.2	0.87	13.7	63.6	0.65	12.8	50.8
	30	2.2	1.3	0.87	14.7	56.9	0.44	10.5	41.5
Corsoy 79	20	2.4	1.1	0.84	14.9	57.3	0.59	11.5	51.1
	25	2.0	1.3	0.89	16.2	55.4	0.36	9.6	38.2
	30	2.0	1.1	0.88	15.2	53.7	0.37	9.8	38.0
Oakland	20	2.4	1.1	0.77	14.0	55.4	0.65	13.7	47.3
	25	2.1	1.2	0.81	15.3	53.4	0.43	10.7	41.1
	30	2.1	1.3	0.88	16.5	53.2	0.36	10.0	36.2
LSD[a]		0.34	0.20	0.207	3.25	11.46	0.159	1.99	8.97
LSD[b]		0.26	0.17	0.176	3.21	10.52	0.097	1.67	6.70

[a]0.05 confidence level, to be used for comparing differences between temperatures within the same cultivar.

[b]0.05 confidence level, to be used for comparing differences between cultivars at the same temperature.

Table 5. Anatomical measurements from cross-sections of 5-day-old hypocotyls of Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean grown at 20, 25, or 30 C

				Pith			Cortex		
Cultivar	Temp.	Diam.	V. C. Diam.	Diam.	Cell No.	Cell size	Width	Cell No.	Cell size
Amsoy 71	20C	2.4mm	1.2mm	0.86mm	15.1	58.0μm	0.63mm	13.2	48.2μm
	25	2.7	1.2	0.94	15.0	60.0	0.74	12.8	58.1
	30	2.3	1.3	0.87	17.4	60.4	0.46	10.6	45.0
Beeson 80	20	2.4	1.0	0.85	13.4	56.0	0.69	13.7	50.5
	25	2.4	1.2	0.94	13.7	63.6	0.65	12.8	50.8
	30	2.2	1.3	0.93	14.7	56.9	0.44	10.5	41.5
Corsoy 79	20	2.4	1.1	0.89	14.9	57.3	0.59	11.5	51.1
	25	2.0	1.3	0.85	16.2	55.4	0.36	9.6	38.2
	30	2.0	1.1	0.82	15.2	53.7	0.37	9.8	38.0
Oakland	20	2.4	1.1	0.77	14.0	55.4	0.65	13.7	47.3
	25	2.1	1.2	0.81	15.3	53.4	0.43	10.7	41.1
	30	2.1	1.3	0.88	16.5	53.2	0.36	10.0	36.2
LSD[a]		0.34	0.20	0.189	3.25	11.46	0.159	1.99	8.97
LSD[b]		0.26	0.17	0.161	3.21	10.52	0.097	1.67	6.70

[a]0.05 confidence level, to be used for comparing differences between temperatures within the same cultivar.

[b]0.05 confidence level, to be used for comparing differences between cultivars at the same temperature.

Table 6. Anatomical measurements from cross-sections of 6-day-old hypocotyls of Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean grown at 20, 25, or 30 C

				Pith			Cortex		
Cultivar	Temp.	Diam.	V. C. Diam.	Diam.	Cell No.	Cell size	Width	Cell No.	Cell size
Amsoy 71	20C	2.3mm	1.2mm	0.87mm	14.8	58.4μm	0.53mm	10.6	49.9μm
	25	2.6	1.3	0.83	15.5	61.4	0.62	11.3	54.9
	30	2.2	1.2	0.90	15.8	55.0	0.48	11.1	45.0
Beeson 80	20	2.4	1.2	0.84	13.9	61.8	0.62	11.5	53.7
	25	2.5	1.4	0.90	15.4	60.8	0.54	11.3	48.0
	30	2.4	1.3	1.03	15.1	61.5	0.50	10.1	48.5
Corsoy 79	20	2.4	1.2	0.95	15.2	59.0	0.58	11.0	53.3
	25	2.0	1.3	0.83	16.4	55.5	0.38	9.7	40.0
	30	2.0	1.2	0.89	16.1	51.6	0.34	8.7	39.4
Oakland	20	2.3	1.2	0.87	14.6	59.7	0.57	12.3	46.7
	25	2.2	1.3	0.80	14.9	58.4	0.43	10.3	42.3
	30	2.0	1.3	0.97	17.1	53.0	0.36	9.8	37.7
LSD[a]		0.34	0.27	0.207	3.97	14.47	0.146	1.85	9.91
LSD[b]		0.19	0.15	0.176	3.32	9.36	0.083	1.87	6.17

[a]0.05 confidence level, to be used for comparing differences between temperatures within the same cultivar.

[b]0.05 confidence level, to be used for comparing differences between cultivars at the same temperature.

Table 7. Anatomical measurements from cross-sections of 7-day-old hypocotyls of Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean grown at 20, 25, or 30 C

				Pith			Cortex		
Cultivar	Temp.	Diam.	V. C. Diam.	Diam.	Cell No.	Cell size	Width	Cell No.	Cell size
Amsoy 71	20C	2.3mm	1.3mm	0.95mm	16.4	57.8μm	0.44mm	9.9	44.2μm
	25	2.3	1.4	1.01	18.0	56.5	0.44	10.2	42.9
	30	2.1	1.3	0.96	17.7	54.5	0.40	10.2	39.5
Beeson 80	20	2.2	1.2	0.82	15.3	53.4	0.50	11.2	44.5
	25	2.4	1.4	0.89	14.1	63.1	0.46	9.5	49.1
	30	2.2	1.4	0.99	18.0	55.3	0.40	9.9	40.4
Corsoy 79	20	1.9	1.2	0.88	17.4	50.7	0.37	9.6	38.7
	25	1.9	1.2	0.83	16.4	51.0	0.33	9.4	35.5
	30	2.2	1.3	1.01	17.7	56.7	0.40	9.7	41.1
Oakland	20	2.4	1.3	0.95	16.7	57.5	0.51	10.9	46.6
	25	2.0	1.3	0.90	17.3	52.0	0.35	9.5	36.2
	30	2.1	1.3	0.95	19.3	49.9	0.36	9.7	37.5
LSD^a		0.37	0.25	0.257	3.80	9.63	0.101	1.54	8.22
LSD^b		0.31	0.22	0.219	3.23	9.27	0.081	1.31	7.00

[a]0.05 confidence level, to be used for comparing differences between temperatures within the same cultivar.

[b]0.05 confidence level, to be used for comparing differences between cultivars at the same temperature.

Cross-section diameter

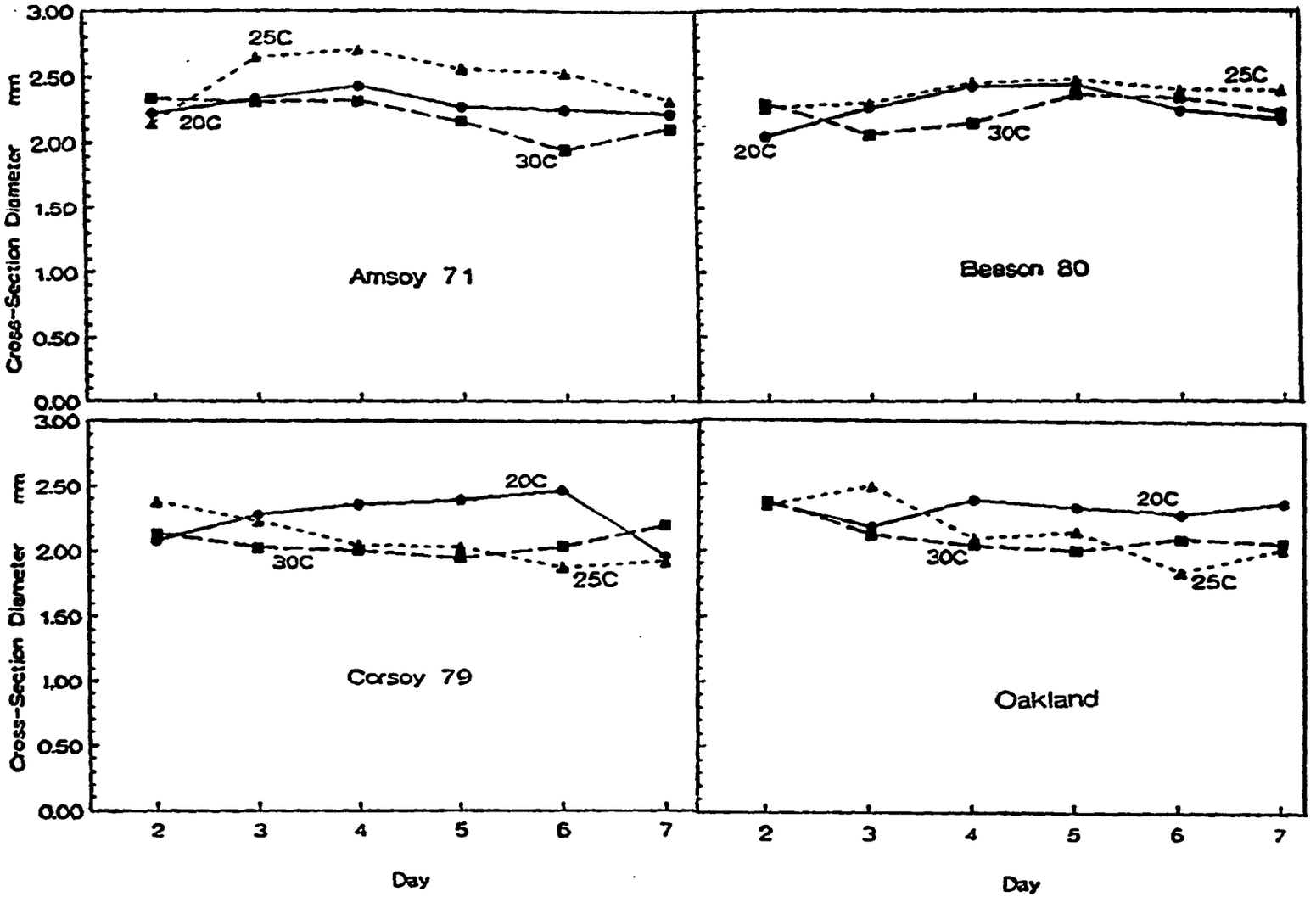
Diameter of cross-sections of hypocotyls collected on days two through seven and measured using an ocular micrometer are presented in Figure 7. Micrometer values were converted to mm by a calibration factor. Day and all interactions involving days were significant at the 0.01 level, as were temperature, cultivar, and the temperature x cultivar interaction (Appendix Table A8). On a daily basis, cultivar and temperature x cultivar were significant at the 0.01 level at days three through seven (Appendix Table A9). Temperature was similarly significant at days two through five.

As can be seen in Figure 7, the general progression is for diameters to be smaller as temperature is increased; however, in most cases, differences between consecutive temperature increments are not significantly different. In Beeson 80 and Oakland, for example, at no day are differences among temperatures significant; however, the trend mentioned above is apparent. There were no significant differences between temperatures in any cultivar at days two and three (Tables 2 and 3, respectively).

In Amsoy 71 and Corsoy 79, day four represents an important time for diameter (Figure 7 and Table 4). In Amsoy 71, diameter at 25 C is significantly greater than at 30 C, but not 20 C. In Corsoy 79, 20 C diameters are greater than

Figure 7. Time of course of cross-section diameter from hypocotyls of Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean grown at 20, 25, or 30 C

Standard errors (mm) are: Day 2=0.57, Day 3=0.15, Day 4=0.15, Day 5=0.11, Day 6=0.14, Day 7=0.16


Cross-Section Diameter mm
3.00
2.50
2.00
1.50
1.00
0.50
0.00
25C
20C
30C
Amsoy 71
Beeson 80
Corsoy 79
Oakland
2
3
4
5
6
7
Day

those at 25 or 30 C. These results are similar to the separation in hypocotyl length among temperatures in these cultivars (Figure 3).

The major apparent difference between this method of determining hypocotyl diameter versus calculation from length and weight data is most noticeably in the early portion of the curves, up to about day three. It appears that determination of hypocotyl diameter by direct measurement tends to result in higher values than the calculated version in the first few days; however, differences between the two methods diminish during the latter half of the seven day period. The significance of these apparent differences will be discussed in a later section.

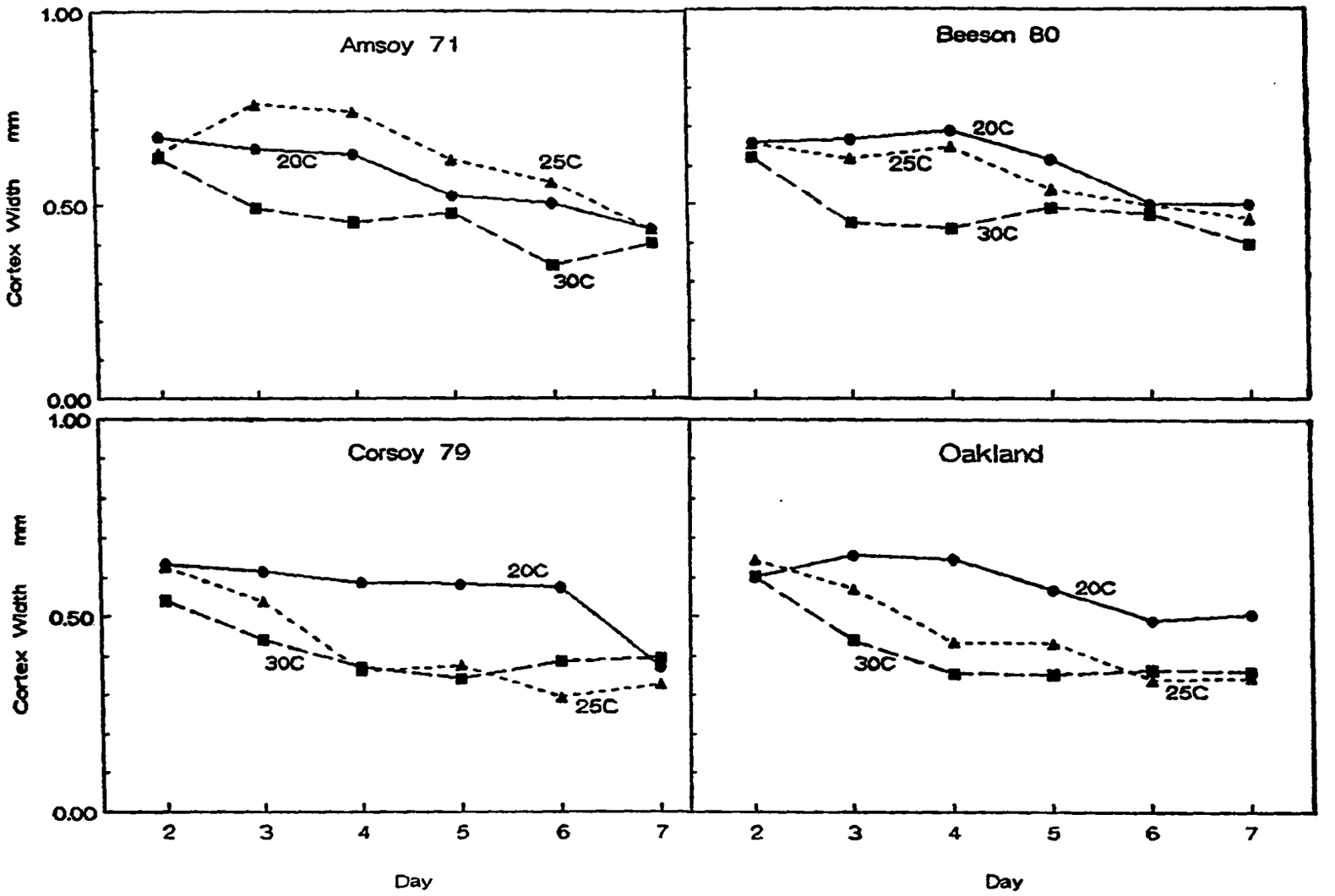
Cortex width

Analysis of variance of cortex width indicated that temperature, cultivar, day, and all interactions were significant at the 0.01 level (Appendix Table A10). Cultivar and the temperature x cultivar interaction were significant at the 0.01 level (Appendix Table A11). Temperature was significant at the 0.01 level at days three through six, and at the 0.05 level at day seven.

There appears to be a general trend in all temperature x cultivar combinations for cortex width to decrease gradually from day two through day seven (Figure 8; see also Tables 2

Figure 8. Time course of cortex width of Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean hypocotyls grown at 20, 25, or 30 C

Standard errors (mm) are: Day 2=0.06, Day 3=0.05, Day 4=0.06, Day 5=0.05, Day 6=0.05, Day 7=0.04


Amsoy 71
Beeson 80
Corsoy 79
Oakland
20C
25C
30C
Cortex Width mm
1.00
0.50
0.00
2
3
4
5
6
7
Day

through 6). It should be noted that in each cultivar the temperature promoting the greatest diameter also results in the greatest cortex width. In the long hypocotyl cultivars, Corsoy 79 and Oakland, 20 C cortex width is significantly greater than that at 30 C from day two through day six, and greater than that at 25 C from day three through day five. In Beeson 80, 20 and 25 C were never significantly different from each other; however, both were greater than 30 C at days three and four. In Amsoy 71, cortex width at 25 C was significantly greater than at 30 C at days three, four, and six. It appears to be greater than at 20 C from days three through six; however, this was not statistically significant.

Cortex cell number and size

Number of cortex cells along a transect through the section were recorded on both sides of the vascular cylinder. Thus, the number of observations in the reported values are double those of the measurements of cross-section diameter, vascular cylinder diameter, and pith. The same is true for values of cortex cell size, which were calculated from the cortex width divided by the number of cells and multiplied by a calibration factor to obtain μm.

Day, temperature, cultivar, and all interactions were significant at the 0.01 level for cortex cell number (Appendix Table A12), and the same was true for cell size

(Appendix Table A14). Temperature, cultivar, and temperature x cultivar were significant at the 0.01 level at days two through seven for cortex cell number (Appendix Table A13). Cultivar and temperature x cultivar were significant at the 0.01 level at days two through seven for cortex cell size (Appendix Table A15). Temperature was not significant at day two; but was significant at the 0.01 level at days three through six, and at the 0.05 level at day seven.

Cortex cell number (Figure 9) and size (Figure 10) were used to explain changes and differences in cortex width. In Amsoy 71, cortex cell number is significantly lower at 30 C than at 20 and 25 C at days three and four (Tables 3 and 4, respectively). A similar response was noted in Beeson 80. This is the same time at which the 30 C cortex width is significantly less than that at the cooler temperatures in these two cultivars. Cell size of Amsoy 71 at 25 is significantly greater than both 20 and 30 C at day four (Table 4); and appears to be greater at days two, five and six; however, the latter observations are not statistically significant (Tables 2, 5, and 6). Temperature effects on cell size in Beeson 80 cannot be statistically separated; however, the 30 C cell size appears to be less than at the other two temperatures at days three and four.

In Corsoy 79, the significantly greater cortex width at days four and five (Figure 8) are largely accounted for by a

Figure 9. Time course of number of cortex cells along a transect through a cross-section of Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean hypocotyls grown at 20, 25, or 30 C

Standard errors are: Day 2=0.86, Day 3=0.80, Day 4=0.95, Day 5=1.07, Day 6=0.88, Day 7=0.67

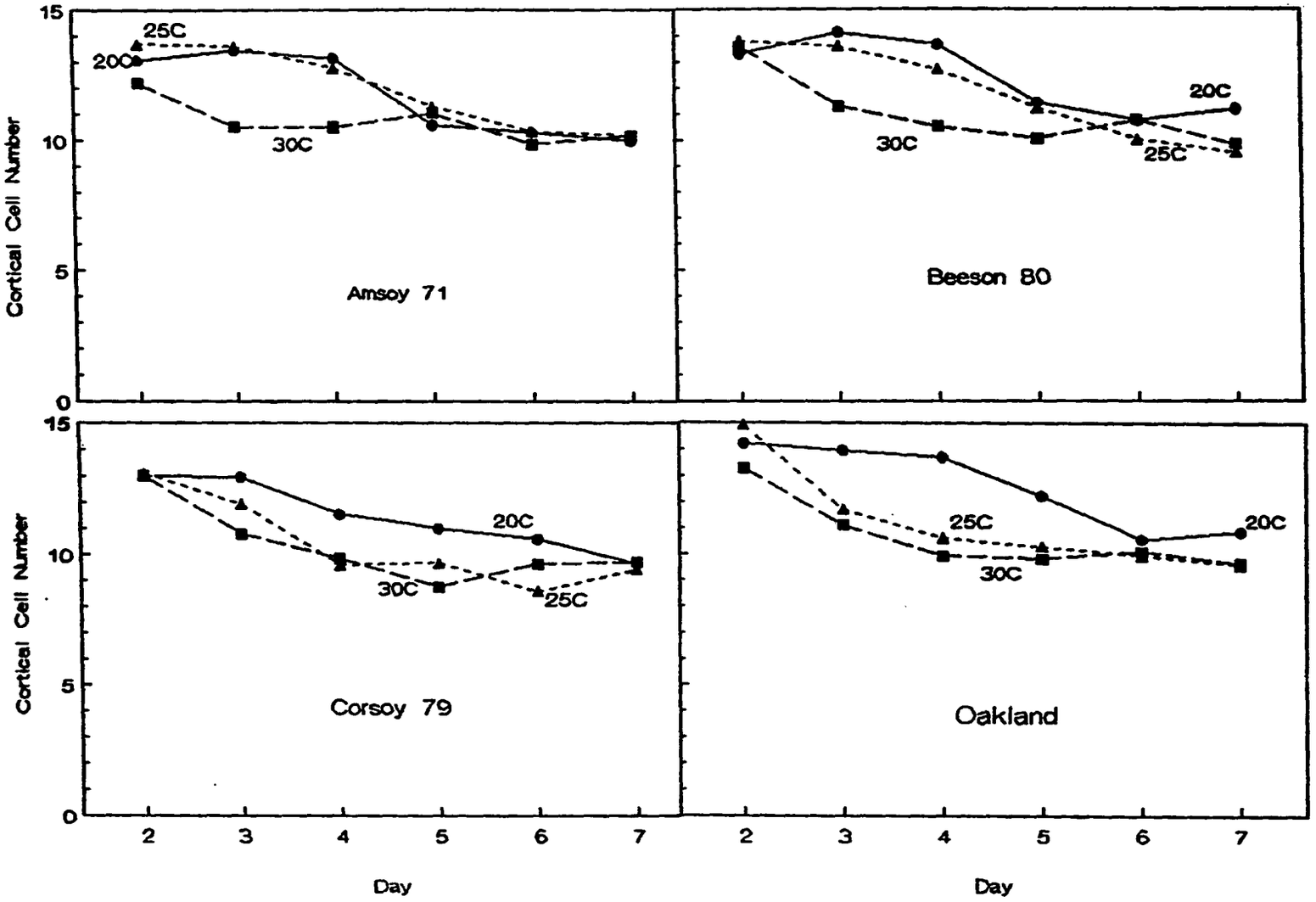
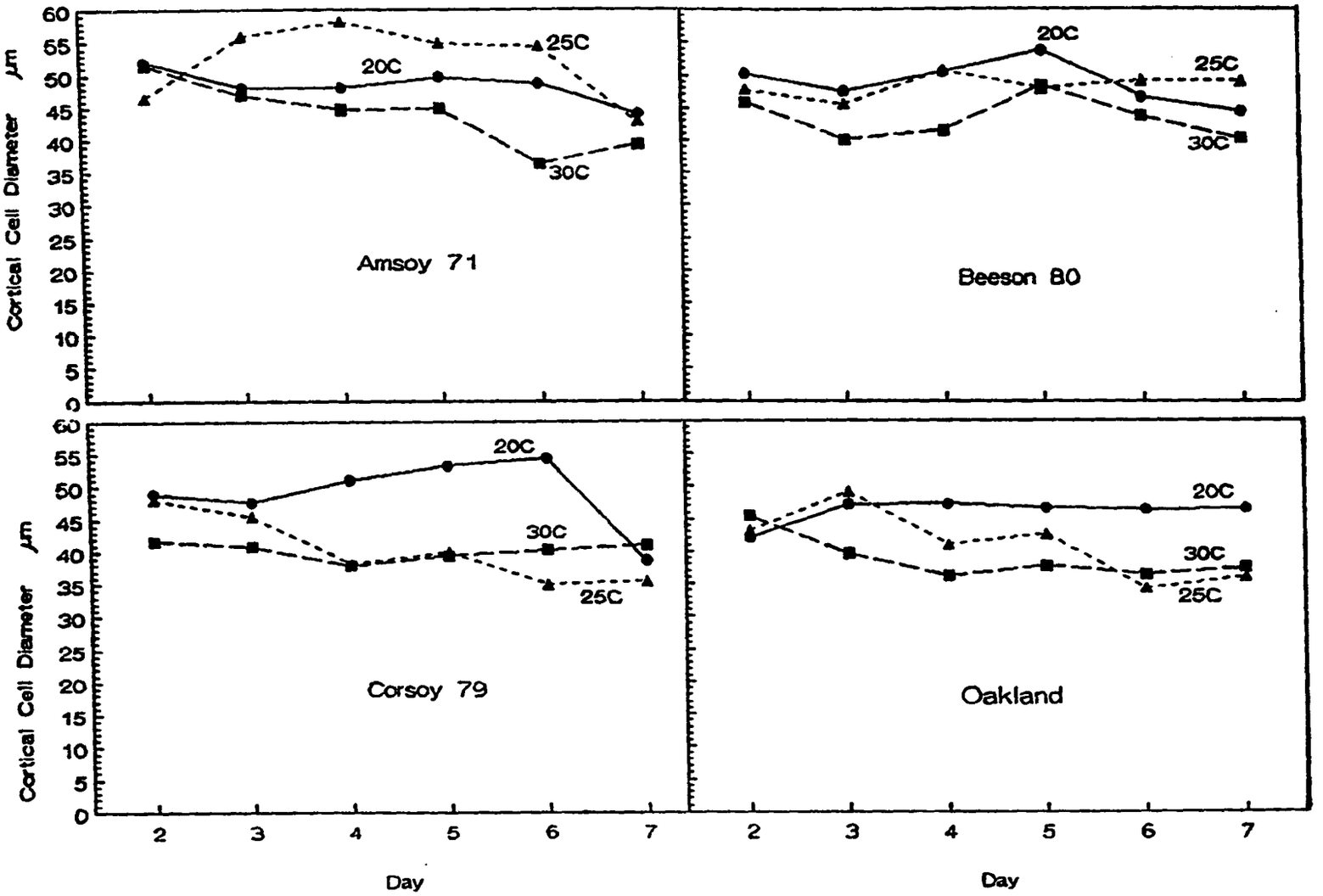

Cortical Cell Number
15
10
5
0
25C
20C
30C
Amsoy 71
Beeson 80
Corsoy 79
Oakland
2
3
4
5
6
7
Day

Figure 10. Time course of cross-sectional cell size of cortex cells of Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean hypocotyls grown at 20, 25, or 30 C

Standard errors (μm) are: Day 2=3.36, Day 3=3.41, Day 4=3.82, Day 5=3.52, Day 6=4.22, Day 7=3.57


Cortical Cell Diameter µm
60
55
50
45
40
35
30
25
20
15
10
5
0
20C
25C
30C
Amsoy 71
Beeson 80
Corsoy 79
Oakland
2
3
4
5
6
7
Day

significantly greater cell size at 20 C at that time. Cell number is not statistically different in response to temperature in Corsoy 79 at days four and five (Tables 4 and 5, respectively). In contrast, Oakland cortex cell number appears to be more closely associated with temperature differences in cortex width. As with Corsoy 79, the greatest cortex width is seen at 20 C during the middle portion of the seven day period; however, in Oakland it is the cell number at 20 C which is significantly greater than at the other two temperatures rather than the cell size. Cell size at 20 C in Oakland is significantly greater than at 30, but not 25 at days four and five.

Vascular cylinder diameter

Diameter of the vascular cylinder was measured along the same transect as cross-section diameter and cortex measurements, and is reported in mm. Day and all interactions involving day were significant at the 0.01 level, as was temperature (Appendix Table A16). Cultivar was not significant; however, the temperature x cultivar interaction was significant at the 0.05 level. When days were analyzed separately, temperature was significant at the 0.01 level at days two, three, and four; but temperature was not significant at day five or later (Appendix A17). Cultivar was significant at the 0.01 level at days two

through six, and at the 0.05 level at day seven. Temperature x cultivar was significant at the 0.01 level at all days.

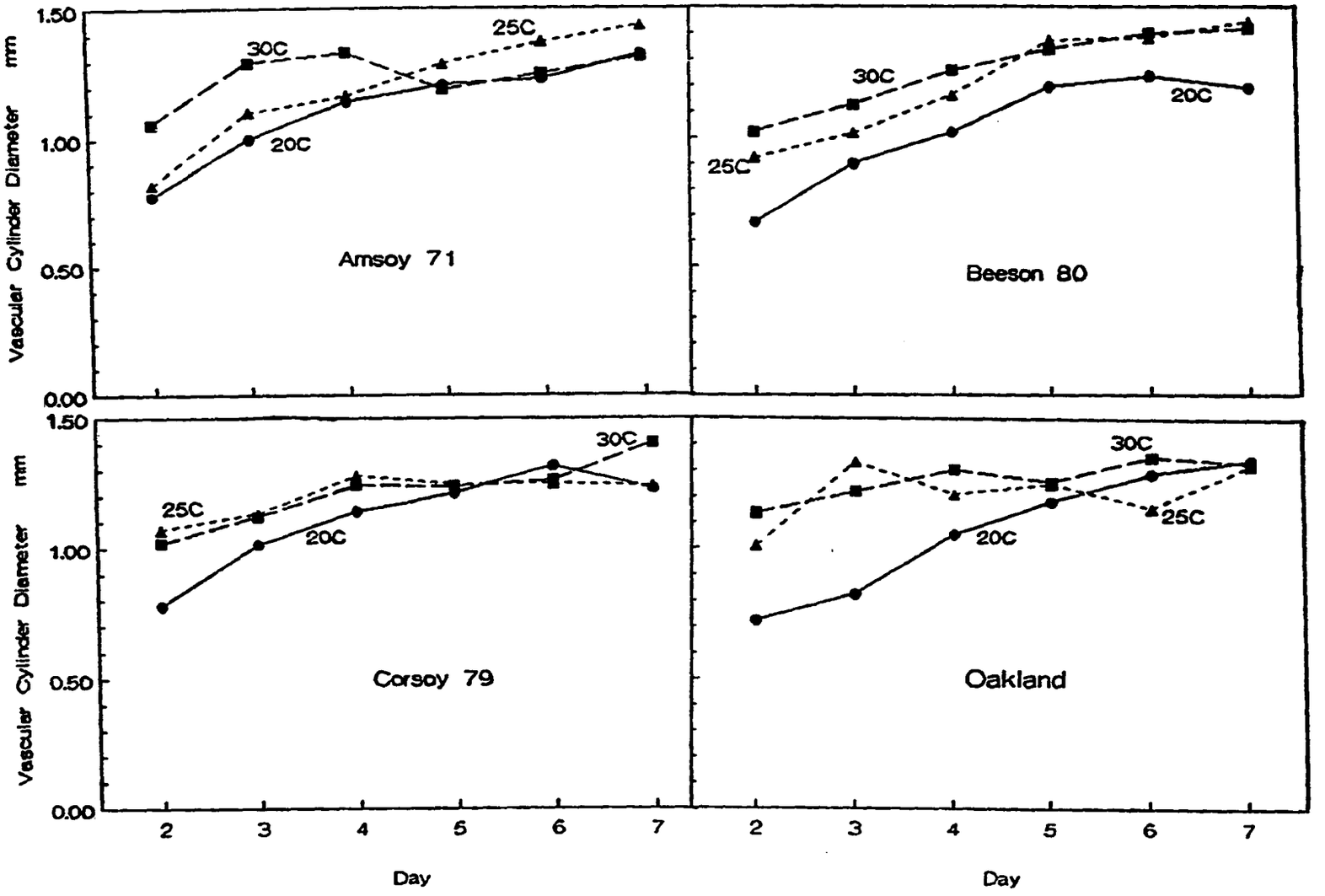
In general, vascular cylinder diameter was greatest at 30 C and least at 20 C in each cultivar (Figure 11; see also Tables 2, 3 , and 4), and in almost all temperature x cultivar combinations, vascular cylinder diameter appears to increase with time. Significant differences among temperatures did not occur after day four in any cultivar, and are rare up to day four. In Amsoy 71, 30 C was significantly greater than 25 and 20 C at day two, but only greater than 20 C at day three. In Beeson 80, 30 C was greater than 20 C, but not 25 C; and at day four, 25 C and 30 C were not significantly different from each other, but both were greater than 20 C. In Corsoy 79, 20 C was significantly lower than 30 C, but not 25 C, at day two. In Oakland, 20 C diameter was significantly less than 25 and 30 C at days two and three, but only different from 30 C at day four.

Pith diameter

Diameter of the pith region was measured along the same transect as previously described for vascular and cross-section diameters, and is reported in mm. Temperature, cultivar, day, and interactions involving day were significant at the 0.01 level (Appendix Table A18). Temperature x cultivar was significant at the 0.05 level.

Figure 11. Time course of vascular cylinder diameter of Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean hypocotyls grown at 20, 25, or 30 C for seven days

Standard errors (mm) are: Day 2=0.08, Day 3=0.08, Day 4=0.10, Day 5=0.09, Day 6=0.08, Day 7=0.11

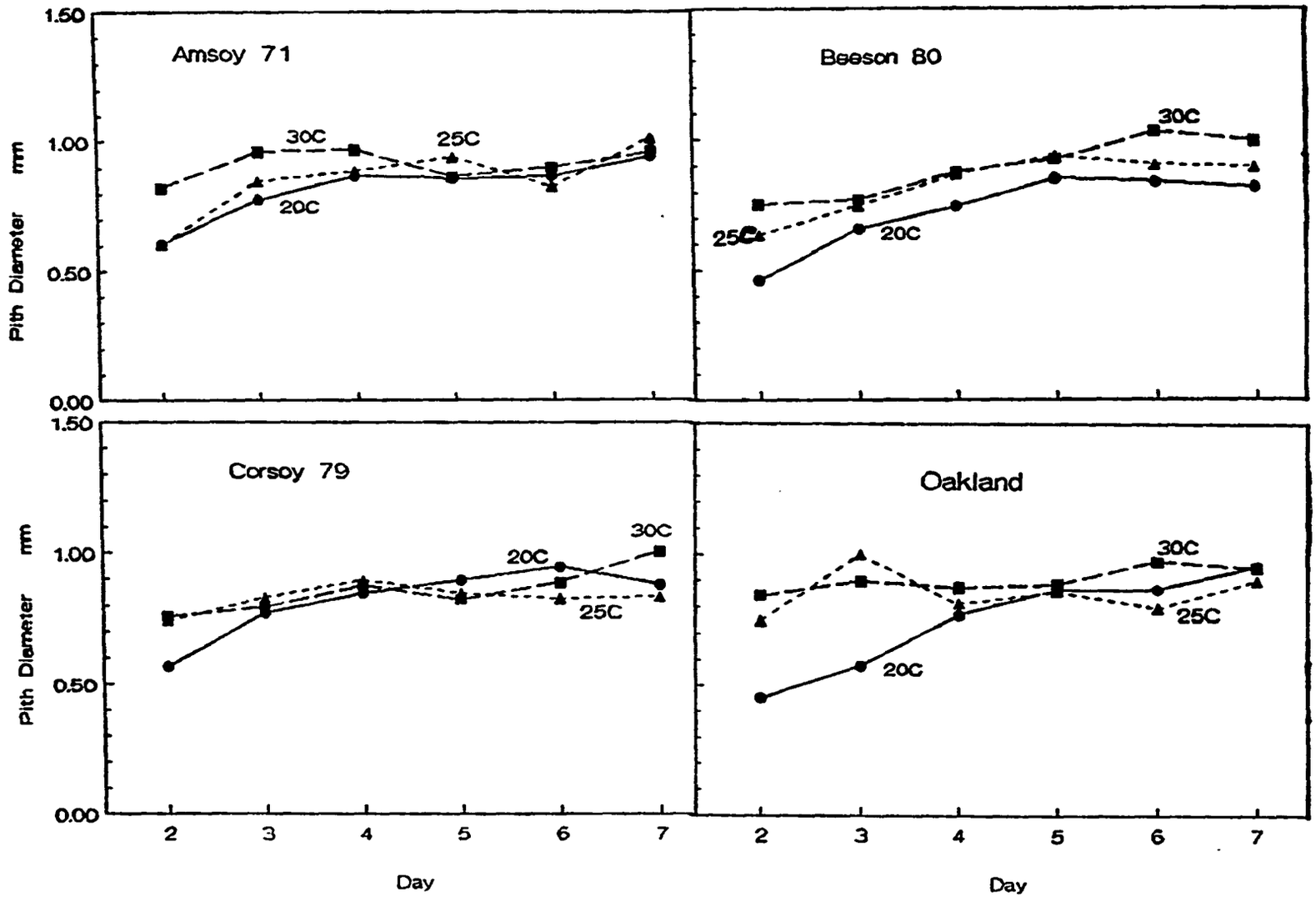

Vascular Cylinder Diameter mm
1.50
1.00
0.50
0.00
30C
25C
20C
Amsoy 71
Beeson 80
Corsoy 79
Oakland
2
3
4
5
6
7
Day

When analyzed on a daily basis, temperature was significant at the 0.01 level at days two, three, and four; and at the 0.05 level at day six (Appendix Table A19). Cultivar was significant at the 0.05 level at day six, at the 0.01 level at days two, four, six, and seven, and not significant at day three. Temperature x cultivar was significant at the 0.01 level at days two, five, six, and seven; but, was not significant at days three and four.

Pith diameter follows the same general pattern as vascular cylinder diameter. Significant differences due to temperature, when present, occurred at times when significant differences occurred for vascular cylinder diameter. Significant differences due to temperature occurred in each cultivar at day two (Table 2), but this was the only day when significant differences could be detected (Figure 12). In Amsoy 71, 30 C pith diameter was significantly greater than 25 and 20 C at day two. At day two in Beeson 80, 20 C pith diameter was significantly less than both of the higher temperatures, which were not different from each other. In Corsoy 79 and Oakland, response to temperature was similar to Beeson 80. The portion of the cross-section that is vascular tissue can be determined by subtracting the pith diameter from the vascular cylinder diameter. This was found not to change significantly over time within temperatures.

Figure 12. Time course of diameter of the pith region of Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean hypocotyls grown at 20, 25, or 30 C

Standard errors (mm) are: Day 2=0.05, Day 3=0.08, Day 4=0.09, Day 5=0.08, Day 6=0.09, Day 7=0.11


Amsoy 71
Beeson 80
Corsoy 79
Oakland
30C
25C
20C
Pith Diameter mm
1.50
1.00
0.50
0.00
2
3
4
5
6
7
Day

Pith cell number and cell size

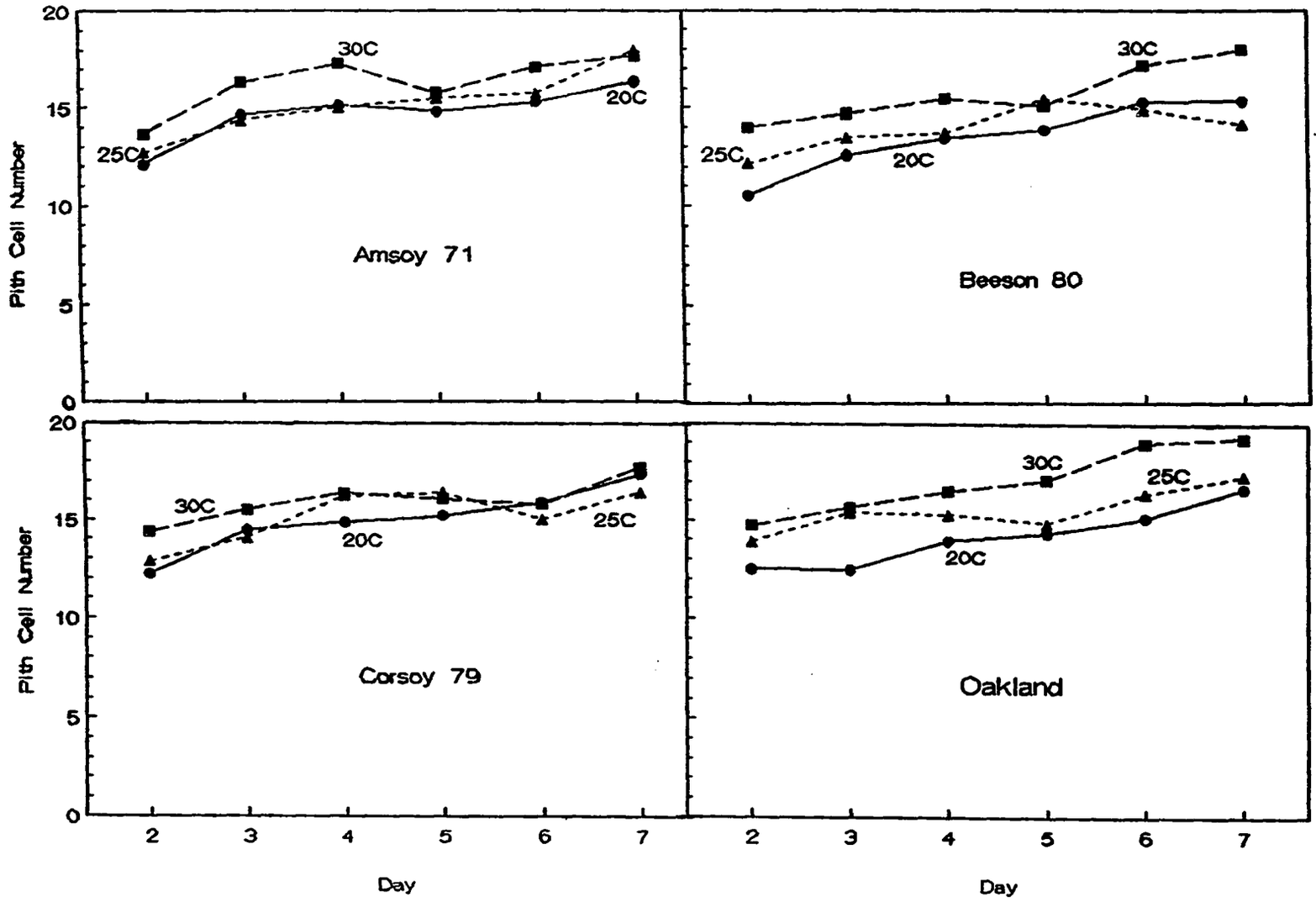
Analysis of variance for pith cell number indicated that temperature, cultivar, and day were significant at the 0.01 level (Appendix Table A20). Interactions of temperature x cultivar and temperature x day were significant at the 0.05 level. Other interactions involving day were significant at the 0.01 level. When days were analyzed separately, temperature was significant at the 0.01 level at all days except day five (Appendix Table A21). Cultivar was significant at the 0.05 level at days five and seven, and at the 0.01 level at other days. Temperature x cultivar was not significant at days two, four, and five, but, was significant at the 0.01 level at days three, six, and seven.

Although a general trend is apparent indicating that pith cell number increases with increasing temperature, no significant differences can be noted (Figure 13). Pith cell number does; however, follow the pattern of pith vascular cylinder diameters of increasing with time at all temperatures.

Pith cell size was determined in a similar manner as cortex cell size and is also reported here in μm. Temperature was significant at the 0.05 level (Appendix Table A22). All other effects and interactions were significant at the 0.01 level. Temperature was significant at the 0.01 level at days two and three, but was not significant at later

Figure 13. Time course of number of pith cells along a transect through a cross-section of Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean hypocotyls grown at 20, 25, or 30 C

Standard errors are: Day 2=1.27, Day 3=1.13, Day 4=1.83, Day 5=1.89, Day 6=1.43, Day 7=1.65

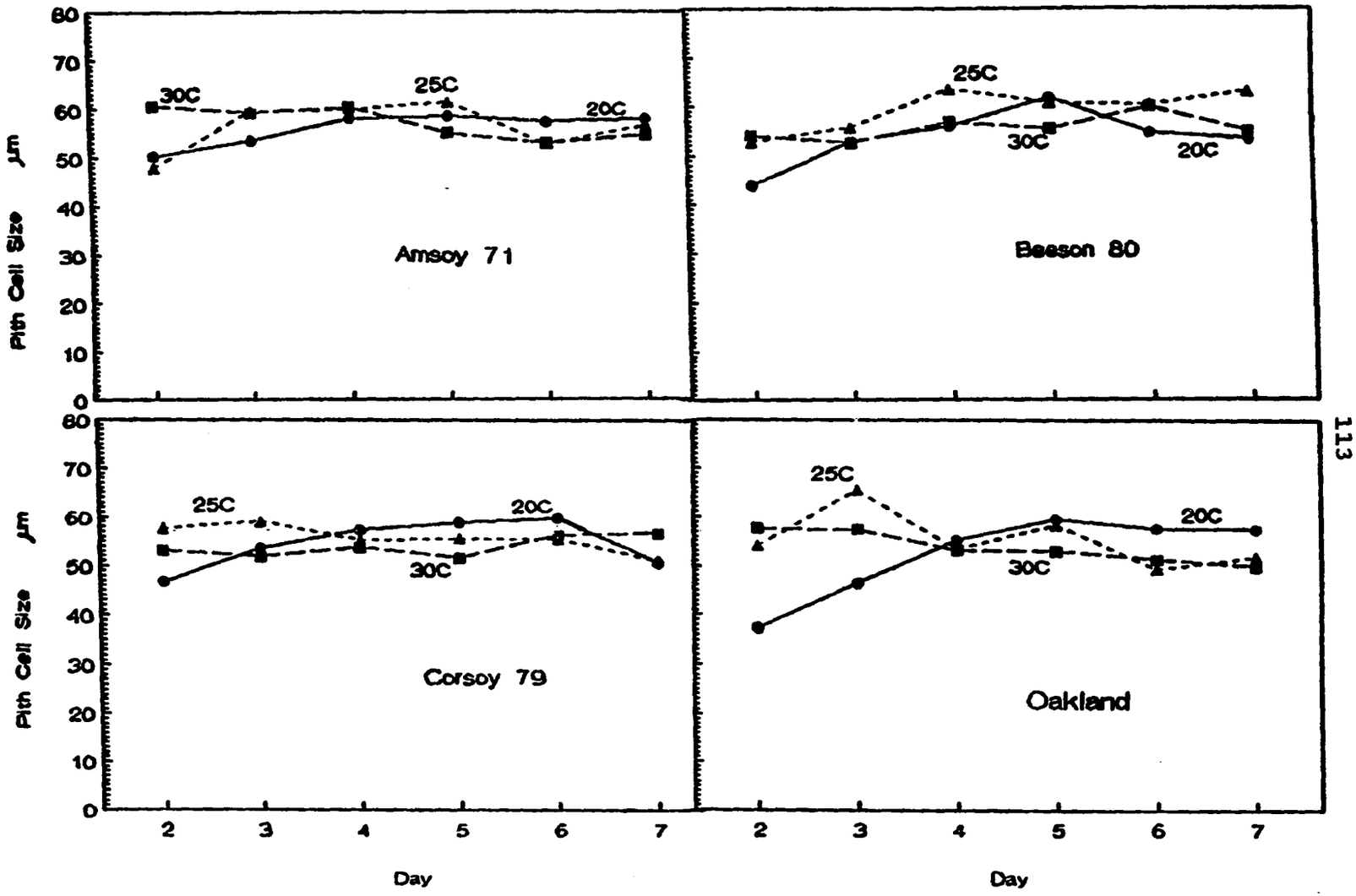

Pith Cell Number
Amsoy 71
Beeson 80
Corsoy 79
Oakland
30C
25C
20C
Day
0
5
10
15
20
2
3
4
5
6
7

days (Appendix Table A23). Cultivar was significant at all days. Temperature x cultivar was significant at 0.05 at days two, three, and six; and at 0.01 at days four, five, and seven.

There were no significant temperature differences in any cultivar for pith cell size after day four, and only in Oakland was there a statistically significant difference at day three (Figure 14 and Table 3). In Oakland, at day three, 25 C pith cell size was significantly greater than 20 C, but not 30 C, and 30 C was not significantly greater than 20 C. In Amsoy 71, 30 C pith cell size was statistically greater than 20 C, but not 25 C; 25 and 20 C were not significantly different. There were no significant differences in Corsoy 79 or Beeson 80 at any day.

Figure 14. Time course of cross-sectional cell size of pith cells from Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean hypocotyls grown at 20, 25, or 30 C

Standard errors (μm) are: Day 2=4.51, Day 3=5.48, Day 4=6.00, Day 5=5.34, Day 6=4.48, Day 7=4.73


Pith Cell Size μm
Amsoy 71
Beeson 80
Corsoy 79
Oakland
30C
25C
20C
Day
0
10
20
30
40
50
60
70
80
2
3
4
5
6
7

DISCUSSION

Inhibition of hypocotyl elongation in Amsoy 71 and Beeson 80 induced by exposure to 25 C was not as severe as was expected on the basis of their previous classification as short hypocotyl cultivars (Seyedin, 1981). There are several possible explanations for this. There may have been a seed quality effect. The seed lots used were produced in the summer of 1983 which was hot and dry during the later part of the season. This could have affected some process or processes related to the general vigor of the seed, as well as the sensitivity of these short hypocotyl types to 25 C inhibition.

Another possibility is the method of production of seedlings and their handling during measurement and sampling procedures; however, much care was taken to use consistent methods for preparing towels, surface sterilization, rolling of towels, manipulation of material under dim green safe-lights, etc., in order to minimize variability. Some seedlings developed abnormally because they shifted position in the towel after it was rolled up, thus altering the gravitational and physical resistance profile of the system. Such seedlings were removed from towels and were not included in measurements. In addition, towels that contained seedlings which exhibited disease symptoms were removed to

avoid further contamination of other seedlings in the same plastic buckets. Furthermore, fungal or bacterial contamination could have adversely altered the nutritional and hormonal balance of the seedlings which would have significantly increased the uncontrolled variability of the experiments, had they been allowed to remain. When there was suspicion that the growth chamber temperature had changed by more than ± 2 or 3 C, experiments were discarded.

The major difference in handling of specimens between this and previous related studies (Seyedin, 1981; Keys, 1979) was the daily measurement of hypocotyl length. The fact that towels were opened daily, and the disturbance of seedlings during the measurement procedure, may have adversely affected seedling elongation. This still may have allowed the specimens to be exposed to temperatures different from the treatment temperature. The temperature of the room where measurements were made varies but is usually within the 20 to 30 C range of temperatures used in these studies. Nevertheless, this possibility is not highly likely because all seedlings used for measurement of hypocotyl length were handled in a similar manner, were only removed from growth chambers for short periods of time each day, and periodic germination tests in which length was measured only at day seven indicated that hypocotyl length was similar to that observed in the experiments. Furthermore, Samimy (1970) was

able to detect inhibited hypocotyl elongation in Clark under conditions where towels were opened on a daily basis.

Despite the lack of a striking elongation inhibition response to 25 C from the short hypocotyl cultivars, some information about the relationship of radial expansion and elongation in soybean hypocotyls in response to temperature can be drawn from results of these studies. These include the temporal correspondence of changes in rate of elongation to changes in hypocotyl diameter, and the spatial relationship of the cortex and pith region contributing to radial expansion.

Length of exposure to a particular temperature regime is important to the degree of response in soybean hypocotyls, as was described by Gilman et al. (1973) in regard to the severity of hypocotyl elongation inhibition in several soybean cultivars at 25 C. This helps to explain why the 25 C hypocotyl length curve of Amsoy 71 was similar in slope to the 20 and 30 C curves up until day three before it began to flatten out, and the actual hypocotyl length neared that of Amsoy 71 at 20 C, as well as the speculation that 25 C hypocotyl length would have been lower than 20 C had the seedlings been allowed to continue growing for a few more days.

The similarity of the 25 C growth anomaly in soybean with the inhibition of elongation and accompanying increased

radial expansion in etiolated pea epicotyls exposed to ethylene was the basis of previous research on the effects of ethylene on the soybean hypocotyl system (Seyedin, 1981; Keys, 1979; Samimy, 1970). The etiolated pea epicotyl is the most closely related species to soybean that has been extensively studied in regard to elongation/expansion; although, there has been some research recently reported for mung bean hypocotyls. In etiolated pea epicotyls exposed to exogenous ethylene, Eisinger et al. (1983) described a change in direction of expansion from longitudinal to radial within three hours after exposure. And Goeschl et al. (1966) reported that ethylene levels increased and pea epicotyls curved in response to physical impedance within six hours following imposition of the stress. Taken together, these results support the suggestion of Knittle (1977) that the increase in radial expansion induced by temperature or by physical stress occur by different mechanisms, although ethylene may be involved in both. Furthermore, the results of this study, together with the results of Gilman et al. (1973) and Samimy (1970) suggest that the mechanism of the 25 C-induced inhibition of short hypocotyl cultivars requires a prolonged exposure to 25 C for the inhibition to occur, and that some factor involved in the induction of the ethylene-producing system may be the time limiting step.

The idea that the inhibition of elongation is a

redirection or redistribution of growth rather than an inhibition of growth per se is indicated by comparing the patterns of percent dry matter (Figure 4), hypocotyl length (Figure 3) and cross-section diameter (Figure 7). Percent dry matter differences among temperatures are slight to nonexistent once the imbibition and germination period has been completed, between days two and three. This corresponds to the time at which significant differences, or at least trends in the direction of, differential responses in hypocotyl length and diameter occur. As mentioned in a previous section, the diameter was generally greater as temperature decreased while hypocotyl length was lessened. This point was suggested by Seyedin (1981); however, he did not report on changes in rate of elongation or diameter over time. Samimy (1970) had earlier reported that the inhibition of elongation in Clark and Shelby was the result of a diversion of dry matter supplied by the cotyledons toward the radicle and away from the hypocotyl. A redistribution of growth from longitudinal to radial was reported to largely account for the inhibition of elongation in the etiolated pea epicotyls treated with ethylene (Eisinger et al., 1983; Burg et al., 1971; Apelbaum and Burg, 1971). It is proposed that this is the case in the soybean hypocotyl because of the lack of change in percent dry matter in response to temperature (Figure 4), and that when hypocotyl length was less radial

expansion increased (Figures 3 and 7).

Hypocotyl diameter was determined by two different methods. The rationale behind this was to attempt to compare the usefulness of determination of hypocotyl diameter by calculation from length and weight data versus the direct measurement of cross-sections of hypocotyl diameter. By comparing Figures 6 and 7 it appears that the calculation method may yield lower values for hypocotyl diameter at day two. At subsequent days; however, there was little difference in the two methods, and variability was similar. The usefulness of the two methods, therefore, would depend on what other parameters were to be compared with the diameter data. In the case of a vigor test, for example, the calculation of hypocotyl diameter from weight and length data would probably be more appropriate since measurements would probably be taken after four or seven days, and length and weight data would be readily available from root and shoot measurements. For the purposes of this study; however, the direct measurement of cross-sections is more useful because the results are being compared to measurement of tissues within the cross-section.

In the etiolated pea epicotyl, the outer portion of the cortex is the tissue responsible for the increase in radial expansion in response to treatment with exogenous ethylene (Taiz, 1984; Stewart et al., 1974). The epidermis has been

shown to be important in mediating the elongation response to auxin (Masuda and Yamamoto, 1972). The proximity of the outer cortex cells to the epidermis may then be important, both for the transport of plant hormones to outer cortex cells, as well as influencing the growth of cortex cells by physically restricting or removing physical restriction from the outer cells of the cortex. Cortex width generally follows the same temporal pattern of response as section diameter (Figures 8 and 7, respectively) and it can be proposed that in the soybean hypocotyl this region is largely responsible for changes in hypocotyl diameter, as is the case in the etiolated pea stem. This would be expected in both cases because the outer cortex cells would not be as physically restricted as more internal cells; such as those along the inner portion of the cortex, or in the pith; however, in this study inner and outer cortex cells were not measured separately.

The conclusion discussed in the preceding paragraph is illustrated by the 25 C cortex width curve in the Amsoy 71 graph (Figure 8). Although not significantly different from 20 C at any day, the 25 C cortex width is significantly greater than that at 30 C at days three, four, and six (Tables 3, 4, and 6). These were the greatest diameters in any cultivar at any day and temperature. Cortex width and section diameter can be compared in the Tables mentioned.

The increase in cortex width occurs at the same time as the increase in diameter at 25 C, and is also the same time at which the slope of the 25 C hypocotyl length curve begins to flatten and approach the 20 C curve. This is indicative of a reduction in rate of elongation, and the corresponding increase in diameter points to a redirection of growth toward radial expansion.

The redirection of growth can also be seen in cultivars which would be expected to respond linearly to temperature. In Corsoy 79 and Oakland, hypocotyl length increases with increasing temperature (Figure 3). At the same time, hypocotyl diameter (Figures 6 and 7) and cortex width decrease with increased temperature and is most evident at days four and five. Diameter and cortex width at 20 C are significantly greater than at 25 and 30 C in both Corsoy 79 and Oakland on those days (Tables 4 and 5). In comparison with hypocotyl length (Figure 3, this is the same time at which 20 C hypocotyl length in Corsoy 79 is significantly lower than 25 and 30 C, and in Oakland the separation between hypocotyl length curves among temperatures are increasingly evident.

The question arises as to whether cell number or cell size of the cortex region changes in response to temperature. On this point, the results of these studies are mixed. In Corsoy 79, cortex cell diameter is more closely aligned with

the significantly greater cortex width and hypocotyl diameter at 20 C than cortex cell number. In Oakland; however, the reverse appears to be the case.

In Amsoy 71 and Beeson 80, cortex cell number at 20 and 25 C were never significantly different from one another (Figure 9); however, both were greater than cortex cell number at 30 C at days three and four. In Beeson 80, this observation appears to be sufficient to account for differences observed in cortex width on days three and four (compare Figure 7 and 9, and Tables 3 and 4), and there was no significant difference among temperatures. In Amsoy 71; however, the differences between cortex width at 20 and 25 were greater than just the increase in cell number. Cortex cell diameter at 25 C is significantly greater than at 20 C at day three and appears to be greater at subsequent days (Figure 10). That increase in cortex cell size corresponds with an increase in cortex width and hypocotyl diameter at 25 C in Amsoy 71 which would agree with the ethylene-treated etiolated pea epicotyl results. It is likely that the increases in diameter that Seyedin (1981) observed in Amsoy 71 hypocotyls in response to exposure to 25 C, or treatment with exogenous ethylene at 30 C, as well as those noted by Knittle (1977) in other short hypocotyl cultivars which respond to physical resistance were due to increases in cortex cell diameter.

The gradual increase in vascular cylinder diameter in all four cultivars appears to be a significant occurrence (Figure 11); however, changes which are apparent in the diameter of the vascular cylinder are mostly due to a similar gradual increase in pith diameter (Figure 12). If values for pith diameter are subtracted from the vascular cylinder diameter, the diameter of the hypocotyl cross section taken up by vascular tissue is not altered within cultivars. It should be noted here that the pith and vascular cylinder diameters increase with time. This increase contributes to the overall increase in diameter of the hypocotyl over the first three to four days observed at all temperatures (Figure 7); however, the lack of significant differences among temperatures within cultivars for pith diameter does not support the idea that pith diameter contributes to differences in hypocotyl diameter among temperatures within cultivars.

Pith cell number (Figure 13) and pith cell size (Figure 14) follow closely the overall increase with time at all temperatures. Furthermore, the lack of difference among temperatures within cultivars for pith diameter is reflected in the pith cell number and size graphs.

In conclusion, the results of these studies supports the idea that the 25 C growth anomaly of short hypocotyl cultivars of soybean, such as Amsoy 71, is due to a

redirection of growth of the cortex cells: a decrease in longitudinal expansion and an increase in radial expansion, and that it is this region of the hypocotyl anatomy which contributes most greatly to the differential response among temperatures within cultivars. Furthermore, the increase in elongation of cultivars whose hypocotyl elongation rate is normally constant is accompanied by a decrease in hypocotyl diameter, which is again due to changes in the cortex. Although the pith region contributes to general growth of the hypocotyl, it does not contribute to differential response in hypocotyl diameter among temperatures within cultivar, regardless of short or long hypocotyl classification. The usefulness of determining hypocotyl diameter by calculation from length and weight data versus direct measurement is dependent on the intended use of the data; however, the calculation method may underestimate hypocotyl diameter for the first one or two days after planting.

SUMMARY

The inhibition of hypocotyl elongation and increase in radial expansion in certain soybean [Glycine max (L.) Merr.] cultivars at 25 C that have normal hypocotyl elongation at higher or lower temperatures has been studied from agronomic, plant breeding, and physiological perspectives. The present study was initiated to: (1) characterize the anatomical development of cultivars sensitive to this growth anomaly, (2) describe the timing of changes in hypocotyl anatomy and the relationship of these changes to the inhibition of elongation, and (3) compare the anatomical development of hypocotyls from 25 C sensitive cultivars to hypocotyls of cultivars which are not sensitive to 25 C inhibition.

Two "short hypocotyl" cultivars, Amsoy 71 and Beeson 80, and two "long hypocotyl' cultivars, Corsoy 79 and Oakland, were selected on the basis of known physiological and morphological characteristics and their emergence scores in field trials. Seedlings of these cultivars grown in rolled towels at 20, 25, or 30 C were sampled at days one through seven after planting to determine hypocotyl length, and percent dry matter. Hypocotyl swelling index (g freshweight/cm) and hypocotyl diameter were calculated from these data.

Cross-sectional diameter, cortex width, vascular

cylinder diameter, pith diameter, and cell number and size of the pith and cortex regions were measured along a transect across the middle of cross-sections from an area of the hypocotyl immediately below the hypocotyl hook. Hypocotyls collected on days two through seven from each temperature-cultivar combination were fixed and stored in FAA until hand sectioning and examination.

Inhibition of elongation in the short hypocotyl cultivars at 25 C was not as severe as expected on the basis of previous studies. There was; however, a temporal relationship between hypocotyl elongation, hypocotyl diameter, and cortex width. As hypocotyl length increased in response to increased temperature, there was an associated decrease in diameter and width of the cortex. Size of cortex cells, and to some degree cortex cell number, were responsible for changes in cortex width in response to temperature which accounted for changes in cortex width and hypocotyl diameter. Alterations in hypocotyl diameter and length were most noticeable at days three and four after planting. At this time, the inhibition of elongation and increase in diameter of Amsoy 71 at 25 C was most evident, as were the linear responses to temperature among the other cultivars. Pith diameter contributed to the overall increase in diameter over time in all cultivars, but was not important in the differences in hypocotyl diameter among temperatures

within each cultivar.

In comparing the determination of hypocotyl diameter by calculation using length and weight data versus direct measurement of cross-sections, it was concluded that the intended use of the diameter data would determine which was more appropriate; however, the calculation method may underestimate diameter in hypocotyls less than three days old.

In the development of seedling vigor tests, the possible influence of an anomalous growth pattern such as this may adversely affect the ability of a laboratory test to predict field performance. The combination of hypocotyl length and radial expansion data would provide an additional screening method in breeding soybeans for high emergence potential and seedling vigor by aiding in the identification of short hypocotyl genotypes.

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APPENDIX

Table A1. Seed lot information for Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean used in these studies[a]

Cultivar	Producer	Lot #	Tested	Purity	Germination	Inert Matter	seed size
Amsoy 71	Sansgaard[b]	0109-31	certification tag missing from bag				152mg
Beeson 80	Sansgaard	0220-30	2/84	99.2%	90%	0.8%	170
Corsoy 79	Sansgaard	33	1/84	99.2	92	0.8	154
Oakland	CAD[c]	31	12/83	99.9	95	0.1	165

[a]Seed weight measured in lab. All other information from Iowa seed certification tag.

[b]Sansgaard Seed Farm, Story City, IA.

[c]Committee for Agricultural Development, Ames, IA.

Table A2. Analysis of variance for hypocotyl length of Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean grown at 20, 25, or 30 C over seven days

Source	df	Mean Square
Rep	1	71.76
Temperature	2	406455.93*
Error A	2	5968.23
Cultivar	3	47148.92*
T x C	6	15742.13
Error B	9	10162.19
Day	6	679616.42**
T x D	12	22518.14**
C x D	18	5355.86**
T x C x D	36	2623.18**
Error C	4104	305.52
Total	4199	2625.07

* Significant at 0.05 confidence level.
** Significant at 0.01 confidence level.

Table A3. Analysis of variance of hypocotyl length by day for Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean grown at 20, 25, or 30 C

Source	df	Day 1	2	3	4
		- - - - - - - - - - Mean Squares - - - - - - - - - -			
Rep	1	6.62	5.04	1156.48	135.38
Temperature	2	39.14**	10981.45	33097.20	78061.81**
Error A	2	0.96	765.22	2594.10	594.14
Cultivar	3	18.32**	3430.56**	3621.01**	6218.26**
T x C	6	3.95**	437.57**	484.16**	2464.57**
Error B	585	1.21	18424.23	98.48	258.11
Total	599	1.45	91.55	240.27	570.76

** Significant at 0.05 confidence level.

Table A3 (continued)

Source	df	Day 5	Day 6	Day 7
		- - - - - - - - Mean Squares - - - - - - - -		
Rep	1	1131.63	665.71	79.935
Temperature	2	18709.21**	158908.91*	171807.04*
Error A	2	760.01	2082.20	1785.13
Cultivar	3	10818.43**	1823.11**	36954.39**
T x C	6	5839.35**	9923.42**	12328.23**
Error B	585	483.03	656.03	757.09
Total	599	985.20	1370.01	1527.54

* Significant at 0.05 confidence level.

Table A4. Analysis of variance for percent dry matter, hypocotyl swelling index (HSI), and calculated hypocotyl diameter (HD) for Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean grown at 20, 25, and 30 C

Source	df	%DM	HSI	HD
		- - - - - - Mean Squares - - - - - -		
Rep	1	12.09	36.39	0.02
Temperature	2	196.99	90.00	0.11
Error A	2	12.04	28.72	0.02
Cultivar	3	36.98	128.43	0.11**
T x C	6	19.79	106.39	0.08**
Error B	9	20.76	13.13	0.01
Day	6	2898.70**	3161.47**	3.68**
T x D	12	71.56**	146.09**	0.13**
C x D	18	10.58	29.04	0.03*
T x C x D	36	38.79	29.67*	0.03*
Error C	72	21.21	17.30	0.01
Total	167	133.00	149.55	0.06

* Significant at 0.05 confidence level.
** Significant at 0.01 confidence level.

Table A5. Analysis of variance for percent dry matter for each of seven days for Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean grown at 20, 25, or 30 C

Source	df	Day 1	2	3	4	5	6	7
		Mean Squares						
Rep	1	121.54	2.86	0.09	1.55	1.35	0.01	0.001
Temperature	2	488.00	100.76	22.30**	11.65	2.58	1.09	0.002
Error A	2	104.74	11.00	0.05	0.80	0.24	0.09	0.16
Cultivar	3	42.24	51.09**	4.65**	0.41	1.14	0.51	0.39**
T x C	6	223.87	24.91**	1.51*	0.99	0.59	0.30	0.37**
Error B	9	148.59	3.44	0.41	1.32	0.38	0.10	0.03
Total	23	178.88	24.35	3.11	1.98	0.75	0.29	0.17

* Significant at 0.05 confidence level.
** Significant at 0.01 confidence level.

Table A6. Analysis of variance for hypocotyl swelling index for each of seven days for Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean grown at 20, 25, or 30 C

Source	df	Day 1	2	3	4	5	6	7
		Mean Squares						
Rep	1	1.78	41.91	5.92	12.41	66.62	19.00	58.66
Temperature	2	75.54**	264.00	373.29	28.32*	64.20	63.89	97.26*
Error A	2	0.69	36.87	65.72	1.41	15.22	22.21	2.54
Cultivar	3	16.01	99.93	106.94	7.86	29.68	15.43	27.32*
T x C	6	6.92	74.60	31.53	34.57*	69.90	40.84	26.05*
Error B	9	5.68	11.01	28.40	8.27	32.18	16.45	4.93*
Total	23	12.82	64.79	71.65	16.41	44.50	27.41	23.52

* Significant at 0.05 confidence level.
** Significant at 0.01 confidence level.

Table A7. Analysis of variance for calculated hypcotyl diameter for each of seven days for Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean grown at 20, 25, or 30 C

Source	df	Day 1	2	3	4	5	6	7
		Mean Squares						
Rep	1	0.01	0.04	0.003	0.01	0.05	0.01	0.04
Temperature	2	0.17*	0.26	0.27	0.02	0.05	0.05	0.07*
Error A	2	0.002	0.04	0.04	0.001	0.01	0.01	0.002
Cultivar	3	0.04	0.10**	0.07*	0.01	0.02	0.01	0.02*
T x C	6	0.02	0.08*	0.02	0.02*	0.05	0.03	0.02*
Error B	9	0.01	0.01	0.02	0.01	0.02	0.01	0.004
Total	23	0.03	0.07	0.05	0.01	0.03	0.02	0.02

* Significant at 0.05 confidence level.
** Significant at 0.01 confidence level.

Table A8. Analysis of variance for cross-section diameter of hypocotyls from Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean grown at 20, 25, or 30 C for seven days

Source	df	Mean Square
Rep	4	0.85
Temperature	2	3.85**
Error A	8	0.36
Cultivar	3	4.36**
T x C	6	1.76**
Error B	36	0.33
Day	5	0.78**
T x D	10	0.93**
C x D	15	0.74**
T x C x D	30	0.57**
Error C	2040	0.15
Total	2159	0.18

** Significant at 0.01 confidence level.

Table A9. Analysis of variance for cross-section diameter for each of seven days for Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean hypocotyls grown at 20, 25, or 30 C

Source	df	Day 2	3	4	5	6	7
		Mean Squares					
Rep	4	0.86	0.03	0.90	0.18	0.20	0.27
Temperature	2	0.44	2.48**	2.32**	1.88**	1.80	0.03
Error A	8	0.95	0.28	0.12	0.16	0.59	0.10
Cultivar	3	0.57	1.13**	2.36**	1.83**	1.20**	1.05**
T x C	6	0.37	0.27**	0.59**	0.66**	1.59**	0.84**
Error B	336	0.66	0.04	0.05	0.02	0.04	0.05
Total	359	0.66	0.08	0.10	0.06	0.10	0.08

** Significant at 0.01 confidence level.

Table A10. Analysis of variance for cortex width of hypocotyls from Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean grown at 20, 25, or 30 C for seven days

Source	df	Mean Square
Rep	4	0.07
Temperature	2	3.36**
Error A	8	0.03
Cultivar	3	1.14**
T x C	6	0.38**
Error B	36	0.02
Day	5	2.36**
T x D	10	0.22**
C x D	15	0.06**
T x C x D	30	0.07**
Error C	2040	0.01
Total	2159	0.02

** Significant at 0.01 confidence level.

Table A11. Analysis of variance for cortex width for each of seven days for Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean hypocotyls grown at 20, 25, or 30 C

Source	df	Day 2	3	4	5	6	7
		Mean Squares					
Rep	4	0.01	0.01	0.13	0.08	0.02	0.03
Temperature	2	0.08	1.30**	1.65**	0.73**	0.59**	0.16*
Error A	8	0.02	0.03	0.03	0.03	0.06	0.02
Cultivar	3	0.05**	0.18**	0.65**	0.31**	0.16**	0.12**
T x C	6	0.02**	0.08**	0.22**	0.13**	0.19**	0.07**
Error B	336	0.01	0.01	0.01	0.01	0.01	0.004
Total	359	0.01	0.02	0.03	0.01	0.02	0.01

* Significant at 0.05 confidence level.
** Significant at 0.01 confidence level.

Table A12. Analysis of variance for cortex cell number of hypocotyls from Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean grown at 20, 25, or 30 C for seven days

Source	df	Mean Square
Rep	4	8.71
Temperature	2	348.31**
Error A	8	1.86
Cultivar	3	113.71**
T x C	6	26.95**
Error B	36	3.53
Day	5	685.49**
T x D	10	47.57**
C x D	15	11.56**
T x C x D	30	8.03**
Error C	2040	1.57
Total	2159	4.12

** Significant at 0.01 confidence level.

Table A13. Analysis of variance for cortex cell number for each of seven days for Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean hypocotyls grown at 20, 25, or 30 C

Source	df	Day 2	3	4	5	6	7
		Mean Squares					
Rep	4	1.59	2.04	14.46	10.72	0.83	2.13
Temperature	2	23.33**	225.75**	235.23**	59.50**	23.41**	18.39**
Error A	8	1.52	2.76	3.56	1.50	4.20	3.08
Cultivar	3	27.18**	20.25**	76.56**	28.41**	13.48**	7.01**
T x C	6	5.71**	12.01**	18.18**	16.60**	5.95**	7.29**
Error B	336	1.48	1.28	1.81	2.28	1.53	0.89
Total	359	1.89	2.91	4.20	3.14	1.88	1.21

** Significant at 0.01 confidence level.

Table A14. Analysis of variance for cortex cell size of hypocotyls from Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean grown at 20, 25, or 30 C for seven days

Source	df	Mean Square
Rep	4	251.04
Temperature	2	7709.19**
Error A	8	89.69
Cultivar	3	4756.38**
T x C	6	1325.41**
Error B	36	621.23
Day	5	1642.86**
T x D	10	470.07**
C x D	15	289.45**
T x C x D	30	437.25**
Error C	2040	28.55
Total	2159	61.00

** Significant at 0.01 confidence level.

Table A15. Analysis of variance for cortex cell size for each of seven days for Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean hypocotyls grown at 20, 25, or 30 C

Source	df	Day 2	3	4	5
		mean squares			
Rep	4	36.09	24.13	212.47	210.55
Temperature	2	175.85	1730.11**	2703.65**	2028.33**
Error A	8	74.69	163.82	89.04	132.02
Cultivar	3	659.25**	706.32**	1638.29**	1435.43**
T x C	6	268.75**	178.28**	709.32**	482.25**
Error B	336	22.15	23.30	29.19	24.78
Total	359	34.12	44.24	72.40	59.83

** Significant at 0.01 confidence level.

Table A15 (continued)

Source	df	Day 6	Day 7
		Mean Squares	
Rep	4	132.64	145.68
Temperature	2	3364.93**	464.73*
Error A	8	324.52	73.01
Cultivar	3	1143.37**	651.12**
T x C	6	11832.21**	495.42**
Error B	336	35.60	25.42
Total	359	90.11	43.36

* Significant at 0.05 confidence level.

Table A16. Analysis of variance for vascular cylinder diameter of hypocotyls from Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean grown at 20, 25, or 30 C for seven days

Source	df	Mean Square
Rep	4	0.10
Temperature	2	5.15**
Error A	8	0.01
Cultivar	3	0.02
T x C	6	0.18*
Error B	36	0.07
Day	5	8.52**
T x D	10	0.42**
C x D	15	0.16**
T x C x D	30	0.18**
Error C	2040	0.02
Total	2159	0.05

* Significant at 0.05 confidence level.
** Significant at 0.01 confidence level.

Table A17. Analysis of variance for vascular cylinder diameter for each of seven days for Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean hypocotyls grown at 20, 25, or 30 C

Source	df	Day 2	3	4	5	6	7
		Mean Squares					
Rep	4	0.06	0.04	0.11	0.02	0.04	0.05
Temperature	2	3.21**	2.24**	1.14**	0.25	0.05	0.34
Error A	8	0.09	0.01	0.03	0.10	0.13	0.12
Cultivar	3	0.18**	0.27**	0.11**	0.11**	0.09**	0.08*
T x C	6	0.17**	0.36**	0.08**	0.05**	0.20**	0.22**
Error B	336	0.01	0.01	0.02	0.02	0.01	0.03
Total	359	0.04	0.04	0.03	0.02	0.02	0.03

* Significant at 0.05 confidence level.
** Significant at 0.01 confidence level.

Table A18. Analysis of variance for pith diameter of hypocotyls from Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean grown at 20, 25, or 30 C for seven days

Source	df	Mean Square
Rep	4	0.08
Temperature	2	2.30**
Error A	8	0.10
Cultivar	3	0.21**
T x C	6	0.18*
Error B	36	0.04
Day	5	3.17**
T x D	10	0.37**
C x D	15	0.11**
T x C x D	30	0.11**
Error C	2040	0.02
Total	2159	0.03

* Significant at 0.05 confidence level.
** Significant at 0.01 confidence level.

Table A19. Analysis of variance for pith diameter for each of seven days for Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean hypocotyls grown at 20, 25, or 30 C

Source	df	Day 2	3	4	5	6	7
		Mean Squares					
Rep	4	0.02	0.002	0.07	0.04	0.03	0.03
Temperature	2	2.24**	1.03**	0.25**	0.03	0.36*	0.21
Error A	8	0.22	0.11	0.03	0.06	0.06	0.07
Cultivar	3	0.10**	0.31	0.16**	0.04*	0.05**	0.10**
T x C	6	0.15**	0.27	0.03	0.05**	0.10**	0.11**
Error B	336	0.01	0.01	0.02	0.01	0.02	0.02
Total	359	0.02	0.03	0.02	0.02	0.02	0.03

* Significant at 0.05 confidence level.
** Significant at 0.01 confidence level.

Table A20. Analysis of variance for pith cell number of hypocotyls from Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean grown at 20, 25, or 30 C for seven days

Source	df	Mean Square
Rep	4	17.55
Temperature	2	676.61**
Error A	8	8.59
Cultivar	3	159.06**
T x C	6	31.95*
Error B	36	9.58
Day	5	689.37**
T x D	10	10.00*
C x D	15	15.32**
T x C x D	30	12.57**
Error C	2040	4.95
Total	2159	7.77

* Significant at 0.05 confidence level.
** Significant at 0.01 confidence level.

Table A21. Analysis of variance for pith cell number for each of seven days for Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean hypocotyls grown at 20, 25, or 30 C

Source	df	Day 2	3	4	5
		Mean Squares			
Rep	4	11.39	4.20	4.85	32.44
Temperature	2	166.22**	122.95**	133.33**	59.43
Error A	8	5.12	11.44	5.40	14.30
Cultivar	3	34.54**	37.99**	52.85**	18.87*
T x C	6	5.70	56.13**	8.71	11.79
Error B	336	3.25	2.56	6.72	7.15
Total	359	4.60	4.02	7.78	8.06

* Significant at 0.05 confidence level.
** Significant at 0.01 confidence level.

Table A21 (continued)

Source	df	Day 6	Day 7
		Mean Squares	
Rep	4	11.49	15.72
Temperature	2	129.66**	116.77**
Error A	8	8.23	12.35
Cultivar	3	28.35**	63.61**
T x C	6	20.37**	29.78**
Error B	336	4.08	5.43
Total	359	5.43	7.22

Table A22. Analysis of variance for pith cell size of hypocotyls from Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean grown at 20, 25, or 30 C for seven days

Source	df	Mean Square
Rep	4	158.93
Temperature	2	1195.27*
Error A	8	217.72
Cultivar	3	975.43**
T x C	6	299.40
Error B	36	168.76
Day	5	1864.63**
T x D	10	1307.45**
C x D	15	355.75**
T x C x D	30	4301.47**
Error C	2040	52.84
Total	2159	75.96

* Significant at 0.05 confidence level.
** Significant at 0.01 confidence level.

Table A23. Analysis of variance for pith cell size for each of seven days for Amsoy 71, Beeson 80, Corsoy 79, and Oakland soybean hypocotyls grown at 20, 25, or 30 C

Source	df	Day 2	3	4	5
		Mean Squares			
Rep	4	57.07	86.89	393.58	169.75
Temperature	2	4441.26**	2119.42**	133.32	691.12
Error A	8	157.34	121.61	92.33	272.40
Cultivar	3	235.21**	264.66**	621.18**	580.92**
T x C	6	754.28**	501.68**	191.93*	134.78*
Error B	336	40.60	60.00	72.02	57.01
Total	359	81.45	82.23	83.02	72.28

* Significant at 0.05 confidence level.
** Significant at 0.01 confidence level.

Table A23 (continued)

Source	df	Day 6	Day 7
		Mean Squares	
Rep	4	144.10	105.84
Temperature	2	220.62	72.29
Error A	8	120.25	104.31
Cultivar	3	602.57**	447.38**
T x C	6	362.25**	541.70**
Error B	336	40.08	44.72
Total	359	53.52	58.55